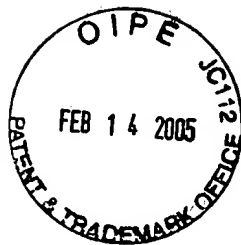


APPEAL BRIEF
SERIAL NO. 08/846,658



CARP0001-100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: Adair et al.

Confirmation No. 9631

Serial No.: 08/846,658

Art Unit No.: 1642

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For: HUMANISED ANTIBODIES

Customer No.: 34132

Docket No.: CARP0001-100

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

APPEAL BRIEF UNDER 37 CFR § 41.37

This is an appeal from the Final Rejection dated as mailed July 12, 2004, rejecting claims 24-31 in the above-identified application. A Notice of Appeal was filed October 12, 2004. A petition for a two-month extension of time and the appropriate fee accompany this brief. The brief fee also accompanies this brief. As February 12, 2005 fell on a Saturday, this brief is timely with the two-month extension.

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REAL PARTY IN INTEREST

The real party in interest is Celltech R & D, Ltd. (formerly Celltech Therapeutics, Limited).

RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences which will directly affect, will be directly affected by, or have a bearing on the Board's decision in the present appeal.

STATUS OF CLAIMS

The claims pending in this application are Claims 24-31. All claims stand rejected. The pending claims are appended hereto in the Claims Appendix.

STATUS OF AMENDMENTS

All amendments have been entered.

SUMMARY OF CLAIMED SUBJECT MATTER

Humanised immunoglobulins are, usually, derived from monoclonal antibodies generated in rodents (mice) that have been genetically engineered to appear more human. The development of monoclonal antibody technology in the late 1970s enabled the preparation of homogeneous antibody populations directed to a single, specific target (page 2, lines 1-3). These "magic bullets," as they are often referred to, had great therapeutic potential. They also had one major drawback – they were generally prepared from mouse sources and administration to humans resulted in a human anti-mouse antibody ("HAMA") response which greatly impaired their effectiveness (page 2, lines 8-29). With the concomitant development of recombinant DNA technology, artisans were able to prepare antibodies that had the binding capabilities of the

mouse monoclonal antibodies yet looked more like human antibodies and, thus, exhibited a decreased HAMA response.

As described in the present specification, early attempts to decrease the HAMA response focused upon matching the entire variable region of the antibody chains, i.e., the region that binds to antigen (page 3, lines 4-16), to the mouse monoclonal antibody. There was still a significant risk of HAMA response (page 3, lines 16-21). Subsequent attempts matched only what are referred to as the Complementarity Determining Regions (“CDRs”) of the variable regions (page 3, lines 22-29) to the mouse monoclonal antibody. These are the regions that are most variable and believed to be responsible for the binding of antigen. Matching of these regions alone, however, was not satisfactory; the binding to antigen was often just a fraction of that of the original monoclonal antibody (page 4, lines 10-14). It was subsequently discovered that having residues match the mouse monoclonal residues in the variable region in addition to the CDRs, i.e., in the framework region, achieved satisfactory binding while minimizing the HAMA response. Initially, two residues outside the CDRs as defined by Kabat et al (Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, NIH, USA, 1987, reference 7 cited on page 65 of the specification), **but within** the structural loops, were also matched to the mouse monoclonal residues, with a resultant improvement in binding over the matching of the CDRs alone (page 4, lines 14-24). The present invention is directed to antibodies having residues **outside** the CDRs as defined by Kabat **and** the structural loops that match the mouse monoclonal residues. The Kabat CDRs completely encompass the structural loops except for the first CDR of the heavy chain. The structural loop for this CDR extends from

residues 26-32 (page 19, lines 28-29); the Kabat CDR extends from residues 31-35 (page 19, line 23).

The claims of the present application are directed to such humanised immunoglobulins.

Support for the limitations of independent claims 24 and 28 in the application as filed is presented in the table below.

24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	Page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1} .	Page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	Page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	Page 6, line 12, to page 7, line 5.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may “contribute to antigen binding” as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are so identified in Figure 4.
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor	Page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

immunoglobulin heavy and light chains	
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity	Page 11, lines 23-30 and page 37, lines 5-10.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	Page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	Page 6, line 12, to page 7, line 5.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may “contribute to antigen binding” as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are so identified in Figure 4.

GROUND OF REJECTION TO BE REVIEWED ON APPEAL

There is one ground of rejection presented for review. The ground of rejection is the rejection of all pending claims under 35 U.S.C. § 102(e) (see Final Rejection, dated July 12, 2004, page 2) as allegedly anticipated by U.S. Patent No. 5,585,089 issued to Queen et al. (“the Queen patent”).

ARGUMENT

Claims 24-31 are not anticipated by the Queen patent (U.S. Patent No. 5,585,089)

Appellants are attempting to provoke an interference with the Queen patent. One ground of rejection of the presently pending claims, i.e., claims 24-31, remains. Claims 24-31 are

rejected under 35 U.S.C. § 102(e) as allegedly anticipated by the Queen patent. For the reasons discussed in more detail below, Appellants maintain that the Queen patent is not an appropriate reference under 35 U.S.C. § 102(e). The Queen patent is not entitled to an effective filing date earlier than Appellants' effective filing date of **December 21, 1989**.

Appellants maintain that the Queen patent is not entitled to the priority dates of its two earliest priority applications filed December 28, 1988 and February 13, 1989, respectively. The next earliest priority date for the Queen patent is **September 28, 1990**, which is after Appellants' effective filing date of December 21, 1989. Because the Queen patent is not entitled to its two earliest priority dates, it is not an appropriate reference under 35 U.S.C. § 102(e).

As has been repeatedly advanced by Appellants during prosecution of the present application, the Queen patent contains several continuation-in-part applications in its priority chain. As has also been repeatedly advanced by Appellants during prosecution of the present application, when the priority chain contains an application that is a continuation-in-part of the parent application

in order to carry back the 35 U.S.C. 102(e) critical date of the U.S. patent reference to the filing date of a parent application, the **>U.S. patent reference< **must** * have a right of priority to the earlier date under 35 U.S.C. 120 or 365(c) and *>the parent application **must**< support the invention **claimed** as required by 35 U.S.C. 112, first paragraph. "For if a patent could not theoretically have issued the day the application was filed, it is not entitled to be used against another as 'secret prior art' under

35 U.S.C. 102(e).” *In re Wertheim*, 646 F.2d 527, 537, 209 USPQ 554,
564 (CCPA 1981).

(MPEP 2163.03, IV, emphasis in bold added.) 35 U.S.C. §112, first paragraph, provides that

The specification shall contain a written description of the invention, and
of the manner and process of making and using it, in such full, clear,
concise, and exact terms as to enable any person skilled in the art to which
it pertains, or with which it is most nearly connected, to make and use the
same . . .

(35 U.S.C. §112, first paragraph.) “Section 112 requires that the application **describe**, enable,
and set forth the best mode of carrying out the invention.” *Festo Corp. v. Shoketsu Kinzoku
Kogyo Kabushiki Co.*, 535 U.S. 722, 724, 62 USPQ2d 1705, 1707 (2002) (emphasis added).
Appellants maintain that the issued claims of the Queen patent are not entitled to the filing dates
of, at least, the two earliest priority applications because there is no written descriptive support
for several of the claim limitations in those applications.

The Queen patent is attached as Evidence Appendix A. Each one of the claims of the
Queen patent contain the following limitations that Appellants have maintained do not find
support in, at least, the two earliest priority documents:

1. “an affinity constant of at least 10^7 M^{-1} ,”
2. “no greater than about four-fold that of the donor immunoglobulin;” and
3. “outside the Kabat and Chothia CDRs.”

See, for example, the Request for Reconsideration filed on May 24, 2000 (“May 2000 Request,”
copy attached as Evidence Appendix B). The May 2000 Request includes a chart appended

thereto as Appendix A identifying, *inter alia*, the foregoing limitations of the Queen patent and setting forth the reasons why they do not find support in the two earliest priority applications. Although most of the exchanges with the Patent Office subsequent to the filing of the May 2000 Request focused upon the last limitation listed above, Appellants have also argued lack of support for the first two limitations.

Addressing each limitation in turn, the recitation of “an affinity constant of at least 10^7 M^{-1} ” is clearly not present in the two earliest priority applications. These applications recite, rather, that the affinity constant is “**stronger** than about 10^8 M^{-1} ” (*see* page 4, line 36, of Application Serial No. 07/310,252, “the 252 application,” Evidence Appendix C and page 4, line 25 of Application Serial No. 07/290,975, “the 975 application,” Evidence Appendix D, emphasis added) and “at least about 10^8 M^{-1} , preferably 10^9 M^{-1} to 10^{10} M^{-1} , or stronger” (*see* page 7, lines 13-14 of the 252 application, Evidence Appendix C and page 8, lines 4-5 of the 975 application, Evidence Appendix D). Claim 13 of the 252 application and claim 5 of the 975 application recite that the affinity be “about 10^8 M^{-1} or stronger” (*see* Evidence Appendix C and Evidence Appendix D, respectively). Claim 12 of the 975 application recites that the affinity is “at least about 10^8 M^{-1} ” (*see* Evidence Appendix D). There is no recitation of an affinity constant of “at least 10^7 M^{-1} ” in these applications.

Nor can it be said that such a recitation is implicit or inherent. The passages cited above are clearly setting a floor for affinity of “about” or “at least about” 10^8 M^{-1} ; the actual affinities are to be greater than this value. The limitation “ 10^7 M^{-1} ,” however, is **not** greater than 10^8 M^{-1} ; it is less. To the extent it can be argued that the recitation of “about” or “at least about” lowers this floor somewhat, it certainly cannot be said that it lowers it 10-fold to include “at least 10^7

M⁻¹.” See *Pieczenik v. Dyax Corp.*, 226 F. Supp. 2d 314, 321 (D.C. Mass. 2002), which stated that:

[i]n similar fashion, in the interest of lexicographic consistency, "at least about 10%" can be understood to perhaps capture 9%, or given the qualification of "at least about 10%," perhaps a number substantially above 10%, but certainly not 1%, as plaintiffs' expert, Dr. Makowski, maintains.

Appellants maintain that there is no written descriptive support for the limitation “at least 10⁷ M⁻¹” in the two earliest priority applications of the Queen patent.

The second limitation listed above, i.e., “no greater than about four-fold that of the donor immunoglobulin,” is also not supported by the two earliest priority applications (*see* chart appended to Evidence Appendix B). This limitation requires that the affinity level should not be four-fold **greater** than that of the donor. As should be apparent from the discussion in the “Summary of Claimed Subject Matter” herein, the concern was not that the affinity levels of the humanized antibodies would be greater than that of the donor immunoglobulin but, rather, that the affinity levels would be much **less** than that of the donor immunoglobulin. Consistent therewith, the 252 application recited that the affinity level be “**within** about 4 fold of the donor immunoglobulin’s original affinity to the antigen” (page 4, lines 35-37, of the 252 application, Evidence Appendix C, emphasis added), suggesting that the affinity level should not be more than four-fold **less** than that of the donor. A review of the 252 application confirms this interpretation. See, for example, the discussion on page 10, lines 7-10, of the 252 application, Evidence Appendix C:

The present invention is based in part on the model that two contributing causes of the **loss** of affinity in prior means of producing humanized antibodies (using as examples mouse antibodies as the source of CDRs) are . . .

Id. (emphasis added). Appellants identified no discussion of this limitation in the 975 application.

Nor can it be said that this recitation is implicit or inherent in either of the priority applications. This recitation is contrary to the expectation in the art for humanized antibodies as compared with the donor antibodies, i.e., a lower affinity. Applicants maintain that there is no written descriptive support for the limitation “no greater than about four-fold that of the donor immunoglobulin” in the two earliest priority applications of the Queen patent.

Finally, as Appellants argued in the Preliminary Amendment filed May 1, 1997 with the present application, and have consistently argued thereafter, the limitation “outside the Kabat and Chothia CDRs” is also not supported by either of the two earliest priority applications. This limitation **requires** that there be changes to donor amino acids in the framework region outside **both** the Kabat and Chothia CDRs. The priority applications did not require that the changes to donor be outside the Chothia CDR.

This limitation was added to the claims in a preliminary amendment in the application that issued as the Queen patent (filed June 7, 1995) and, indeed, was necessary to secure allowance of the Queen patent claims over the prior art. During prosecution of the Queen patent, the Riechmann reference, *inter alia*, was cited to support an obviousness rejection. (See Paper No. 7, Application Serial No. 07/634,278, filed December 19, 1990, attached as Exhibit 6 to the

Supplemental Amendment filed in the present application on March 17, 2003, hereinafter “Supplemental Amendment,” page 7, attached hereto as Evidence Appendix E.) The Riechmann reference discloses an antibody in which the CDRs (as defined by Kabat) and, additionally, residue 27 alone or residues 27 and 30 combined are changed to donor. The Queen claims at the time the rejection was levied recited that the CDRs “and **at least one** residue immediately adjacent to at least one of said CDRs are from different immunoglobulin molecules than the framework regions.” (Paper No. 6, Application Serial No. 07/634,278, filed December 19, 1990, attached as Exhibit 5 to the Supplemental Amendment filed in the present application on March 17, 2003, page 1, emphasis added, attached hereto as Evidence Appendix F.)

As discussed above, the extents of the Kabat CDRs and the Chothia hypervariable loops (hereinafter “Chothia CDRs”)¹ are not the same. For example, for the heavy chain, the first Kabat CDR comprises residues 31-35 (*see* page 19, lines 15-23, of the present application as filed); the first heavy chain Chothia CDR, however, comprises residues 26-32 (*see* page 19, lines 24-30, of the present application as filed). Residue 30, disclosed to be changed to donor in the Riechmann reference, is **immediately adjacent** to the first **Kabat** CDR for the heavy chain, but **within** the first **Chothia** CDR for the heavy chain. Riechmann, thus, read on the limitation that “at least one residue immediately adjacent to at least one of said CDRs are from different immunoglobulin molecules than the framework regions.” To overcome the rejection over Riechmann, Queen ultimately added the limitation that the residue to be changed to donor must

¹ Although the Chothia reference, discussed *infra*, does not call the regions CDRs, but rather hypervariable loops, Appellants are using the patentees’ nomenclature for ease of discussion.

be outside the Kabat **and** Chothia CDRs in a preliminary amendment that accompanied the filing of the application which issued as the Queen patent. The rejection was not applied thereafter.

The two earliest priority applications, however, did not require that there be changes to donor in the framework outside both the Kabat **and** Chothia CDRs. Rather, they described either a single change to donor anywhere in the framework, including residues within the first Chothia heavy chain CDR, i.e., residues 26-32 or, even, no change to donor in the framework. See, for example, claims 8 and 17 and page 21, lines 23-29 – suggesting that residues 27 and 30 be changed to donor, both of which are within the first heavy chain Chothia CDR -- of the 252 application (Evidence Appendix C), and page 21, lines 23-30 of the 975 application – suggesting that residues 27 and 30 be changed to donor, both of which are within the first heavy chain Chothia CDR (Evidence Appendix D). Indeed, the 975 application, which was directed to a specific antibody, contemplated just matching the CDRs (*see* page 8, lines 7-11 of Evidence Appendix D).

Nonetheless, throughout prosecution of the present application, the Office has endeavored to find support for this limitation in the two earliest priority applications. Initially, the Office simply cited to allegedly supporting text in the priority applications without advancing any argument as to why the text supported the limitation. See, for example, the Final Rejection dated as mailed May 28, 1999 (“May 1999 Action”). In the May 1999 Action, the Office argued that the limitation was taught on page 9, lines 1-5, of the 975 application and page 13, lines 1-18 of the 252 application. As Appellants argued in the response thereto, neither passage cited supports the Office’s contention.

The passage on page 9, lines 1-5, of the 975 application (Evidence Appendix D), contains a background discussion of the hypervariable regions, which it reports are also called CDRs. References by Kabat and Chothia are cited, and incorporated by reference. This passage is the only one in the 975 application linking Chothia to the term "CDRs." Other passages specifically referring to the CDRs make it clear that the CDRs are as defined by Kabat. For example, on page 10, line 2, of the 975 application (Evidence Appendix D), the framework regions are defined in terms of Kabat. If the framework regions are defined in terms of Kabat, the CDRs must be as well. On page 21 of the 975 application (Evidence Appendix D), the protocol for selecting which residues in the heavy chain are to be donor is set out. At lines 19-22, residues which fall in positions within a CDR **"as defined by Kabat, [i.e.,] amino acids 31-35 . . ."** are specified to be donor. At lines 28-30, amino acid 30 is listed as a position **immediately adjacent to a CDR** to be changed to donor. Amino acid 30 is adjacent to the first Kabat heavy chain CDR, but **within** the first Chothia heavy chain CDR. The description of Figure 1 of the 975 application indicates that it refers to the heavy chains and that the three CDRs are underlined (page 6, lines 1-6, Evidence Appendix D). In Figure 1, only amino acids 31-35 are underlined for the first heavy chain CDR.

Neither is there support for this limitation in the other passage relied upon by the Office, i.e., page 13, lines 1-18 of the 252 application. The reference to Chothia in this passage is in the context of computer programs for computer modeling of antibodies. There is no reference to CDRs. Contrastingly, the specific references to CDRs in the 252 application make it clear that the CDRs are as defined by Kabat. On page 8, lines 22-26, the 252 application (Evidence Appendix C) reports that the extents of the framework region and CDRs have been "precisely

defined” by Kabat. On page 21, of the 252 application (Evidence Appendix C), the protocol for selecting which residues in the heavy chain are to be donor is set out. At lines 20-22, residues which fall in positions within a CDR “**as defined by Kabat, [i.e.,] amino acids 31-35 . . .**” are specified to be donor. At lines 27-29, amino acid 30 is listed as a position **immediately adjacent to a CDR** to be changed to donor (emphasis added). Amino acid 30 is adjacent to the first Kabat heavy chain CDR, but **within** the first Chothia heavy chain CDR. The description of Figure 1 of the 252 application indicates that it refers to the heavy chains and that the three CDRs are underlined (page 5, lines 13-20). In Figure 1, only amino acids 31-35 of the first heavy chain CDR are underlined. As is clear from the foregoing, all specific references to CDRs in both the 252 application and the 975 application are to Kabat CDRs.

The first time the Office advanced an argument as to why the passages cited in the 975 application and the 252 application supported its interpretation was in an Advisory Action dated as mailed July 31, 2001 (“July 2001 Action”). Rather than argue that the two priority applications supported the limitation “outside the Kabat and Chothia CDRs,” the Office asserted that one of ordinary skill, based on the 975 specification, would have recognized that CDRs as taught by the Queen patent would **include** CDRs as defined by Chothia, regardless what the rest of the specification discloses as examples of Kabat CDRs. Presumably, in the Office’s view, if the specification supported an interpretation of CDRs to mean Kabat and Chothia, the issue for support of the limitation “outside the Kabat and Chothia CDRs” was resolved. This interpretation of the term “CDRs” as found in the claims of the Queen patent, however, is inconsistent with the claims, specification, and file history of the Queen patent, much less, as

discussed above, the two earliest priority documents. As the Court of Appeals for the Federal Circuit has consistently held:

In determining the meaning of disputed claim language, a court looks first to the intrinsic evidence of record, examining, in order, the claim language itself, the specification, and the prosecution history.

Alza Corp. v. Mylan Laboratories Inc., 391 F.3d 1365, 1370, 73 USPQ2d 1161, 1164 (Fed. Cir. 2004), citing *Interactive Gift Express, Inc. v. Compuserve, Inc.*, 256 F.3d 1323, 1331, 59 USPQ2d 1401, 1407 (Fed. Cir. 2001) (citing *Vitronics Corp. v. Conceptronic, Inc.*, 90 F.3d 1576, 1582, 39 USPQ2d 1573, 1576 (Fed. Cir. 1996)).

Claim 1 of the Queen patent is duplicated below:

1. A humanized immunoglobulin having **complementarity determining regions (CDRs)** from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^7 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework **outside the Kabat and Chothia CDRs**, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin

heavy or light chain frameworks, and each of said donor amino acids:

- (I) is adjacent to a CDR in the donor immunoglobulin sequence or
- (II) contains an atom within a distance of 4 Å of a CDR in said humanized immunoglobulin.

(The Queen patent, claim 1, Evidence Appendix A, emphasis added.) The first limitation of claim 1 does not recite “Kabat and Chothia CDRs” but, merely, “CDRs.” Further, the specification of the Queen patent does not define the term “CDRs” as meaning Kabat and Chothia. The specification defines CDRs in terms of Kabat. For example, the general protocol set forth at column 14 of the Queen patent, listing the categories of what amino acids may be selected as donor, defines CDRs in terms of Kabat :

Category 1: The amino acid position is in a CDR is [sic] defined by Kabat et al., op. cit.

(see col. 14, lines 1-2, of the Queen patent, Evidence Appendix A.) Consistent therewith, all the examples in the Queen patent list residues 31-35 as the residues for the first CDR of the heavy chain (see Tables 1, 4, 6, 7, 8, and 9 of the Queen patent, Evidence Appendix A). As discussed above, the first heavy chain Kabat CDR comprises residues 31-35; the first heavy chain CDR of Chothia comprises residues 26-32.

Further, as Appellants pointed out previously during prosecution, such an interpretation would make the recitation “outside the Kabat and Chothia CDRs” superfluous. But, as Appellants also argued, the prosecution history for the Queen patent indicates that this recitation

was necessary to secure allowance. Indeed, when faced with a rejection over Riechmann, Queen did **not** argue that “CDR” means Kabat plus Chothia. Queen, rather, added the “outside the Kabat and Chothia CDRs” limitation in the application that issued as the Queen patent. (*See* the Supplemental Amendment, Evidence Appendix L.) As Appellants also argued therein, Queen submitted a glossary during prosecution of the application that issued as the Queen patent which stated that two distinct definitions of CDRs are in use – Kabat and Chothia. The Office’s interpretation is not supported by the Queen file history.

Additionally, as is clear from the discussion of the two earliest priority documents above, all specific references to CDRs were to Kabat CDRs, not Kabat and Chothia. Accordingly, even if the Office’s interpretation were correct, the two earliest priority documents do not support the Office’s interpretation.

Finally, as Appellants argued previously, the Office’s interpretation was not only contrary to the intrinsic record (as set forth above), it was also contrary to what Queen had argued during prosecution of the European equivalent applications of the Queen patent when faced with rejections/objections similar to the written description requirement of 35 U.S.C. § 112, first paragraph. Notably, the European equivalent applications claimed priority to the 975 application and the 252 application.

In Queen’s European patent 451,216 (“the European patent”), granted claim 1 recited that there was to be “at least one amino acid substitution outside of” CDRs “as defined by Kabat et al . . . together with Chothia et al . . .” (copy attached as Exhibit 1 to the Request for Reconsideration filed May 20, 2002, attached hereto as Evidence Appendix G). The European patent was revoked in its entirety under Article 123(2) of the European Patent Convention, which

was set forth in the Request for Reconsideration filed May 20, 2002 (Evidence Appendix H) and is duplicated below:

A European patent application or a European patent may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed.

The European Board of Opposition (“European Board”) concluded that

the feature *Kabat [...]together with Chothia [...]*” in claim 1 has neither a technically reasonable nor a legal basis in the application documents as filed; claim 1 does not therefore meet the requirements of Art. 123(2) EPC.

(See Interlocutory decision in Opposition Proceedings, page 27, copy enclosed with the Request for Reconsideration filed May 20, 2002, as Exhibit 4, attached hereto as Evidence Appendix I.) Again, the European patent claimed priority to the 975 application and the 252 application. The European Board also interpreted the reference merely to “CDRs” in granted claim 7 of the European patent, without further definition, to mean Kabat and Chothia CDRs and, thus, revoked it as well. *Id.*

In an appeal of the decision revoking the patent, Queen submitted claims similar to granted claim 7, i.e., referencing merely “CDRs.” Queen argued that, contrary to the finding of the European Board,

. . . unless specifically defining CDR’s otherwise as done in granted claim 1, the person skilled in the art when reading the application **as filed** and the patent specification would have

inevitably understood that the CDRs in granted claim 7 referred
to Kabat CDRs.

(See paper filed June 22, 2001 by Protein Design Labs in appeal of EP-B1 0451 216, page 8, copy attached to the Request for Reconsideration filed May 20, 2002, as Exhibit 5, attached hereto as Evidence Appendix J, emphasis added.) Again, the European patent claimed priority to the 975 application and the 252 application.

Indeed, in a paper filed in a separate action, i.e., the opposition of a divisional application stemming from the application that issued as the European patent, Queen further argued that

. . . **nowhere** does the contested Patent state that the Chothia
definition is to be used in **carrying** out the invention or in
understanding the claims.

(See paper filed July 13, 2001 by Protein Design Labs in EP 95 10 5609.2, page 6, copy attached to the Request for Reconsideration filed May 20, 2002 as Exhibit 6, attached hereto as Evidence Appendix K, emphasis added.) Appellants maintained that the Office's position that the term "CDRs" included the Kabat and Chothia CDRs was not only inapposite to the intrinsic record but, also, was inapposite to the interpretation Queen advanced in another forum when faced with a rejection similar to lack of written description for a similar claim term.

The Office argued that the last-cited passage above was not relevant to the specification of the 975 application since it clearly seemed to refer to the added passage in the European patent. The Office had apparently overlooked the fact that the added passage was objectionable precisely because there was no support for it in the very same two priority applications which Appellants contend do not support a similar recitation. The Office was reminded of this in a

response filed December 23, 2002, with a Request for Continued Examination. Nonetheless, in the Office Action that followed that response, dated as mailed March 26, 2003 (“March 2003 Action”), the Office maintained the position that CDRs meant Kabat plus Chothia.

In the interim, the Supplemental Amendment discussed above had been filed by Appellants on March 17, 2003, which had not been received prior to the March 2003 Action. In the Office Action that followed the Supplemental Amendment, dated as mailed August 21, 2003 (“August 2003 Action”), the Office now **changed** its position and, quite surprisingly, argued that Appellants had misquoted the examiner’s previous position. The Office stated that

The Examiner did not state that CDRs means Kabat “plus” Chothia. Rather, the Examiner position is that CDRs, as incorporated by reference by Queen et al in the ‘975 specification could mean **either** the CDR amino acids defined by Kabat, **or** the amino acids in the hypervariable region taught by Chothia et al (The hypervariable regions are also called CDR’s [sic] according to Queen et al, in 07/290975 application, p. 8, last paragraph, bridging p.9).

(See August 2003 Action, sentence bridging pages 3-4, *et. seq.*, emphasis added.) In the immediately prior Office Action, however, the Office had asserted that

one of ordinary skill in the art would have recognized that CDRs as taught by Queen et al would **include also** CDRs as defined by Chothia et al, besides CDRs as defined by Kabat et al, regardless

of whether the rest of the specification discloses as examples

Kabat's CDR's.

(See March 2003 Action, page 4, emphasis added.) This passage would seem to support Appellants' interpretation of the Office's prior position. Regardless, the intrinsic record of the Queen patent does not support this latter interpretation of CDRs either.

The first limitation of claim 1 of the Queen patent does not recite "Kabat or Chothia CDRs" either, but merely "CDRs." (See the Queen patent, claim 1, Evidence Appendix A, emphasis added.) Further, the specification of the Queen patent does not define the term "CDRs" as meaning Kabat or Chothia. Again, the general protocol set forth at column 14 of the Queen patent listing the categories of what amino acids may be selected as donor defines CDRs in terms of Kabat :

Category 1: The amino acid position is in a CDR is [sic] defined by Kabat et al., op. cit.

(see col. 14, lines 1-2, of the Queen patent, Evidence Appendix A.) And, all the examples in the Queen patent list residues 31-35 as the residues for the first CDR of the heavy chain (see Tables 1, 4, 6, 7, 8, and 9 of the Queen patent, Evidence Appendix A). And, as discussed above, the first heavy chain Kabat CDR comprises residues 31-35; the first heavy chain CDR of Chothia comprises residues 26-32. Such an interpretation is inconsistent with the claims and specification of the Queen patent.

Further, such an interpretation would also make the recitation "outside the Kabat and Chothia CDRs" superfluous. But, as discussed above, the prosecution history of the Queen patent indicates that the recitation was necessary to secure allowance over the prior art. Indeed,

when faced with a rejection over Riechmann, Queen did **not** argue that “CDR” means Kabat or Chothia. Queen, rather, added the “outside the Kabat and Chothia CDRs” limitation in the application that issued as the Queen patent. Thus, such an interpretation is inconsistent with the file history.

Finally, as is clear from the discussion of the two earliest priority documents above, all references to CDRs were to Kabat CDRs, not Kabat or Chothia. Accordingly, even if the Office’s interpretation of CDRs were correct, the two earliest priority documents do not support the Office’s interpretation.²

Indeed, as Appellants advanced, the Office’s new interpretation would render the claims indefinite. Depending upon which CDR is contemplated, a product, may or may not infringe. Further, one cannot tell whether the additional limitation of the claims 1 – i.e., “adjacent a CDR in the immunoglobulin sequence”-- is satisfied because the answer will change depending upon the limits of the CDRs.

Appellants arguments, however, were unavailing. The Final Rejection from which Appellants are appealing followed. In that Final Rejection, the Office maintained its position, and alleged that it meant to argue Kabat or Chothia in the earlier March 2003 Action. The Office argued that

. . . dependent which of the definition of CDRs, Kabat or Chothia, is used
in the humanized antibody, it would be routine in the art to determine

² Notably, the Office relied upon the very same passage in the 975 application to support the Kabat **or** Chothia interpretation that it relied upon to support the Kabat **and** Chothia interpretation for the term “CDRs”— page 9, lines 1-5, of Application Serial No. 290,975, Evidence Appendix D.

which amino acids constitute the framework, or which amino acids are outside of or adjacent to the CDRs, since the amino acids of the Kabat or Chothia CDRs are well known in the art.

(Final Rejection dated as mailed July 12, 2004, page 4.) The Office's analysis, however, focuses upon enablement, which is distinct from written description and definiteness. Appellants position is, and has been, that there is no written descriptive support for the limitation "outside the Kabat and Chothia CDRs" in the two earliest priority applications. The Office has not successfully countered this position with its strained interpretations of the term "CDRs;" there is no written descriptive support for those interpretations either.

CONCLUSION

Appellants maintain that there is no support in the two earliest priority applications for at least three limitations recited in all claims of the Queen patent. Those limitations are:

1. “an affinity constant of at least 10^7 M^{-1} ,”
2. “no greater than about four-fold that of the donor immunoglobulin;” and
3. “outside the Kabat and Chothia CDRs.”


The absence of support for any one of these limitations in the two earliest priority applications precludes reliance on those applications for a reference date under 35 U.S.C. § 102(e). *In re Wertheim*, 646 F.2d 527, 209 USPQ 554 (CCPA 1981). The rejection of claims 24-31 under 35 U.S.C. § 102(e) over the Queen patent, thus, is inappropriate and should be reversed.

Respectfully submitted,

Date:

February 14, 2005

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Doreen Yatko Trujillo
Registration No. 35,719

CLAIMS APPENDIX

24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1} , wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.

25. A humanized immunoglobulin according to claim 24 which specifically binds to an antigen with an affinity in the range 10^8 - 10^{12} M^{-1} .

26. A humanized immunoglobulin according to claim 24, wherein the antigen is an IL-2 receptor.

27. A humanized immunoglobulin according to claim 24, wherein the donor immunoglobulin is the anti-CD4 T-cell receptor antibody.

28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.

29. A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity similar to that of said donor immunoglobulin.

30. A humanized immunoglobulin according to claim 28, wherein the antigen is a human CD3 T-cell receptor.

31. A humanized immunoglobulin according to claim 28, wherein the donor immunoglobulin is the anti-CD3 T-cell receptor antibody.

EVIDENCE APPENDIX

INDEX:

Evidence Appendix A – U.S. Patent No. 5,585,089 issued to Queen et al

The Queen patent was initially submitted with the Preliminary Amendment filed concurrently with the present application on May 1, 1997, and was relied upon by the Office in making the rejection being appealed in the Office Action dated as mailed November 16, 1998.

Evidence Appendix B³ -- Request for Reconsideration filed on May 24, 2000 and Appendix A attached thereto

The May 24, 2000 Request for Reconsideration was referenced as considered in the Final Rejection dated as mailed September 8, 2000.

Evidence Appendix C – Queen Application Serial No. 07/310,252

Relied upon by the Office during examination of the present application. See, for example, the Final Rejection dated as mailed May 28, 1999. It was submitted in the parent of the present application, Application Serial No. 08/303,569 (“the 569 application”), with a protest filed by the proprietors of the Queen patent on April 1, 1997. A copy of the protest, and the Office’s reference to same, is attached to this index. The 569 application was withdrawn from issuance for consideration of the protest.

Evidence Appendix D – Queen Application Serial No. 07/290,975

Relied upon by the Office during examination of the present application. See, for example, the Final Rejection dated as mailed May 28, 1999. It was submitted in the parent of the present application, the 569 application, with a protest filed by the proprietors of the Queen patent on April 1, 1997. A copy of the protest, and the Office’s reference to same, is attached to this index. The 569 application was withdrawn from issuance for consideration of the protest.

³ Entry of Evidence Appendix into the record was confirmed upon review of the Image File Wrapper for this application.

Evidence Appendix E -- Paper No. 7, Application Serial No. 07/634,278, filed December 19, 1990, attached as Exhibit 6 to the Supplemental Amendment filed March 17, 2003, page 7.

Arguments supported thereby acknowledged in the Office Action dated as mailed August 21, 2003. Resubmitted to Examiner Davis on February 10, 2005, for entry.

Evidence Appendix F -- Paper No. 6, Application Serial No. 07/634,278, filed December 19, 1990, attached as Exhibit 5 to the Supplemental Amendment filed March 17, 2003, page 1.

Arguments supported thereby acknowledged in the Office Action dated as mailed August 21, 2003. Resubmitted to Examiner Davis on February 10, 2005, for entry.

Evidence Appendix G⁴ -- Queen's European patent 451,216

Copy attached as Exhibit 1 to the Request for Reconsideration filed May 20, 2002. Acknowledged in the Advisory Action dated as mailed August 28, 2002.

Evidence Appendix H⁵ -- Request for Reconsideration filed May 20, 2002

The Request for Reconsideration filed May 20, 2002 was acknowledged by the Office in the Advisory Action dated as mailed August 28, 2002.

Evidence Appendix I⁶ -- Interlocutory decision in Opposition Proceedings for Queen European Patent

Copy attached as Exhibit 4 to the Request for Reconsideration filed May 20, 2002. Acknowledged in the Advisory Action dated as mailed August 28, 2002.

Evidence Appendix J⁷ -- Paper filed June 22, 2001 by Protein Design Labs in appeal of EP-B1 0451 216

⁴ Entry of Evidence Appendix into the record was confirmed upon review of the Image File Wrapper for this application.

⁵ Entry of Evidence Appendix into the record was confirmed upon review of the Image File Wrapper for this application.

⁶ Entry of Evidence Appendix into the record was confirmed upon review of the Image File Wrapper for this application.

Copy attached as Exhibit 5 to the Request for Reconsideration filed May 20, 2002. Acknowledged in the Advisory Action dated as mailed August 28, 2002.

Evidence Appendix K⁸ -- Paper filed July 13, 2001 by Protein Design Labs in EP 95 10 5609.2

Copy attached as Exhibit 6 to the Request for Reconsideration filed May 20, 2002. Acknowledged in the Advisory Action dated as mailed August 28, 2002.

Evidence Appendix L⁹ -- Supplemental Amendment filed March 17, 2003

Referenced in the Office Action dated as mailed August 21, 2003.

⁷ Entry of Evidence Appendix into the record was confirmed upon review of the Image File Wrapper for this application.

⁸ Entry of Evidence Appendix into the record was confirmed upon review of the Image File Wrapper for this application.

⁹ Entry of Evidence Appendix into the record was confirmed upon review of the Image File Wrapper for this application.

DOCKET NO.: CARP-0057

PATENT

RESPONSE UNDER 37 CFR 1.116
EXPEDITED PROCEDURE
EXAMINING GROUP NO. 1642

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658**

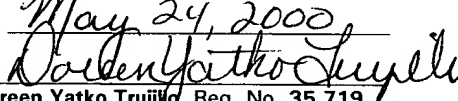
Group No.: **1642**

Filed: **May 1, 1997**

Examiner: **J. Burke**

For: **Humanised Antibodies**

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being transmitted by facsimile to Examiner Burke of the U.S. Patent and Trademark Office, Washington, D.C. 20231.

On May 24, 2000

Doreen Yatko Trujillo Reg. No. 35,719

BOX AF

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

REQUEST FOR RECONSIDERATION

This paper is being filed following the notice of appeal filed November 29, 1999, received by the Patent Office December 1, 1999. Applicants hereby petition for an extension of time of four-months and the undersigned authorizes the Examiner to charge the appropriate fee therefor to Deposit Account 23-3050.

Claims 24-31 were pending. In the Final Rejection, all pending claims were rejected. Amendments following the Final Rejection attempting to add claim 49 were not entered. In an effort to advance the case, claim 49 is not resubmitted herein. Applicants

respectfully submit that allowable subject matter has been identified and request that the interference be declared.

Preliminarily, it is noted that the Examiner stated that the Information Disclosure Statements filed in the parent cases will be considered once the references are submitted. To the extent the Examiner is requiring that Applicants resubmit references already submitted, this appears to be contrary to MPEP § 609, page 600-103. Applicants are not required to resubmit references to get them considered by the Examiner.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 24-31 were again rejected in the Final Rejection under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection for the reasons that follow. For the Examiner's convenience, the paragraphs are designated to correspond to the Examiner's paragraphs for the rejection remaining under this section.

a. and b. The Examiner again rejected claims 24 and 28 alleging that the specification does not provide support for the concept that only substitutions adjacent CDRs are envisaged. Claims 24 and 28 were previously amended to recite that each of the donor amino acids to be replaced is adjacent a CDR or contributes to antigen binding as determined by X-ray crystallography. During a telephone conference between the Examiner and the undersigned, the Examiner indicated that removal of the "adjacent to a CDR" language would

obviate this rejection. Although Applicants disagree with the Examiner's reasoning, the claims have been amended herein to remove the recitation "adjacent to a CDR in the donor immunoglobulin sequence." As Applicants made clear in the previous response, the contribution to antigen binding need not be direct and, indeed, can be indirect, e.g., by affecting antigen binding site topology or inducing stable packing. Naturally, even for an indirect effect, the residues must be spatially near the CDR.

Applicants respectfully request that this rejection be withdrawn.

Rejection Under 35 U.S.C. § 102(e)

Claims 24-31 were again rejected under 35 U.S.C. § 102(e) in view of the Queen patent. Applicants respectfully traverse this rejection. Again, the relevant inquiry as to whether the Queen patent is an appropriate reference under 102(e) is whether there is support for the claims *as allowed* in the priority applications, see MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981), not simply whether the limitations can be found in the priority document. Regardless, Applicants maintain that several of the limitations recited in the claims as allowed in the Queen patent do not find support in the two earliest Queen priority applications – i.e., Queen priority Application Serial No. 07/290,975, filed December 28, 1988 ("Queen '975") and Queen priority Application Serial No. 08/310,252, filed February 13, 1989 ("Queen '252"). For the Examiner's convenience, a chart delineating those limitations for which Applicants submit there is no support in Queen '975 and Queen '252 is attached as Appendix A. The Queen priority applications do not provide a written description of the invention as claimed in the Queen patent. 35 U.S.C. § 112, 1st ¶. The

claims as issued are not completely within the scope of the parent case generic disclosure. *In re Ahlbrecht*, 168 USPQ 293, 296 (CCPA 1971). Thus, the effective filing date of the Queen patent cannot be earlier than September 28, 1990, the next priority date. As evidenced by Appendix C, discussed *infra*, Applicants are entitled to their GB priority date of December 21, 1989. Accordingly, the Queen patent is not an appropriate reference under 35 U.S.C. § 102 (e), and this rejection should be withdrawn.

One of the limitations for which there is no support is the recitation "outside the Kabat and Chothia CDRs." This limitation is significant because the "CDRs" as defined by Kabat and Chothia differ.¹ Kabat defines CDR1 of the heavy chain as amino acids 31-35. Chothia defines the first hypervariable loop of the heavy chain as residues 26-32. This limitation is also significant because it was required for patentability.

In the Final Rejection, the Examiner argued that the limitation "outside the Kabat and Chothia CDRs" is taught, for example, on page 9, lines 1-5 of Queen '975 and page 13, lines 1-8 of Queen '252. The passages cited by the Examiner, however, do not support the Examiner's position.

The passage on page 9, lines 1-5, of Queen '975, contains a background discussion of the hypervariable regions, which it is therein stated are also called the CDRs. References by Kabat and Chothia are cited, and incorporated by reference. This is the only in

¹Notably, Chothia and Lesk, *J. Mol. Biol.*, 196:901-917, 1987, (cited at col. 15, line 43, of the Queen patent for defining CDRs) refers to hypervariable loops and carefully distinguishes these loops from the Kabat CDRs (see page 904 of the Chothia reference, attached).

passage in Queen '975 linking the Chothia reference to the term "CDRs." Other passages specifically referring to the CDRs as encompassed by the invention of Queen '975 make it clear that the CDRs are as defined by Kabat. For example, on page 10, line 2, the framework regions are defined in terms of Kabat. If the framework regions are defined in terms of Kabat, the CDRs must be as well. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 19-22, residues which fall in positions within a CDR **"as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106"** are to be donor. In lines 28-30, amino acid 30 is listed as a position **immediately adjacent to a CDR** to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but **within** the heavy chain Chothia "CDR" as that term is used in Queen '975. The description of Figure 1 of Queen '975 indicates that it refers to the heavy chains and that the three CDRs are underlined (page 6, lines 1-6). In Figure 1, amino acids 31-35 are underlined for CDR1. Clearly, all specific references to CDRs in Queen '975 are to Kabat CDRs only.

The passage relied upon by the Examiner for referring to Chothia in Queen '252 is in the context of computer programs for computer models. There is no reference to CDRs. Contrastingly, the specific references to CDRs in Queen '252 make it clear that the CDRs are as defined by Kabat. On page 8, lines 22-26, Queen '252 reports that the extents of the framework region and CDRs have been "precisely defined" by Kabat. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 20-22, residues which fall in positions within a CDR **"as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106"** are to be donor. In lines 27-29, amino acid 30 is listed as a

position immediately adjacent to a CDR to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but **within** the heavy chain Chothia "CDR" as that term is used in Queen '975. In Figure 1, amino acids 31-35 are underlined for CDR1. Clearly, all specific references to CDRs in Queen '975 are to Kabat CDRs only.

Applicants respectfully request that this rejection be withdrawn.

The Proposed Count is the same as that submitted with the Amendment filed April 9, 1999. Applicants again identify all of the Queen patent claims 1-11 and Applicants' claims 24-31 as corresponding to the Proposed Count.

In attached Appendix B, applicants illustrate the representative support in their present application disclosure for the limitations of their amended claim 24. There is, of course, additional support in applicants' application omitted for the sake of brevity.

In attached Appendix C is a diagram of support in applicants' 1989 GB application for each limitation of applicants' amended claim 28, also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

In view of the foregoing, Applicants respectfully submit that allowable subject matter has been identified and request that the Examiner declare an interference between the

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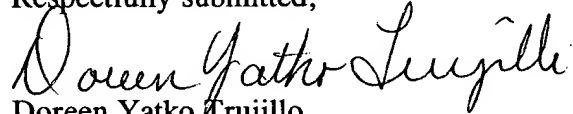
PATENT

present application and the Queen patent.

If at least one of the presented claims is not rejectable on any []
ground and is claiming the same invention as at least one claim of
the patent, the examiner should proceed to initiate an
interference.

MPEP 2307.02. Applicants respectfully request that an interference between the present
application and the Queen patent be declared. The Examiner is requested to contact the
undersigned at (215) 564-8352 if she is of a different view.

Respectfully submitted,



Doreen Yatko Trujillo

Registration No. 35,719

Date: May 24, 2000

WOODCOCK WASHBURN KURTZ
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One Liberty Place - 46th Floor
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APPENDIX A

Claim Limitation	Support in Queen Priority Applications
1. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains,	
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^7 M^{-1}	No. Limitation in bold not recited in two earliest priority applications; recitation sets a different floor for affinity than recited in the priority applications. These applications recite binding affinities "stronger than about 10^8 M^{-1} " (page 4, line 36 of Queen '252 and page 4, line 25 of Queen '975) and "of at least about 10^8 M^{-1} , preferably 10^9 M^{-1} to 10^{10} M^{-1} , or stronger" (page 7, lines 13-14 of Queen '252 and page 8, lines 4-5 of Queen '975). The claims of both applications recite an affinity of 10^8 M^{-1} . The affinities of the example antibodies are not provided; all that is stated is that they have approximately the same affinity as the donor. The affinity of the donor antibody is not provided.

<p>and no greater than about four-fold that of the donor immunoglobulin,</p>	<p>No. Limitation not present in two earliest priority applications. Rather, Queen '252 recites that affinity levels "may be within about 4 fold of the donor immunoglobulin's original affinity to the antigen" (page 4, line 36-37). The background of Queen '252 focuses upon the problem of humanization procedures that result in a loss of affinity for the antigen of at least 2 to 3-fold, and as much as 10-fold (page 2, lines 30-35). Recitation in Queen '252 was, thus, setting a floor for affinity, not a ceiling. The recitation in the issued claims is setting a ceiling. No similar recitation is present in Queen '975.</p>
<p>wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside the Kabat and Chothia CDRs,</p>	<p>No. Limitation not present in two earliest priority applications. Neither application specifies that the antibodies must comprise residues from the donor framework outside the Kabat and Chothia CDRs. CDRs and frameworks are defined in terms of Kabat in both priority applications (page 8, lines 21-25 of Queen '252 and page 21, lines 19-22 of Queen '975). There is no specific reference to or definition of Chothia CDRs. The claims of Queen '252 recite that only a single residue need be changed (claim 8). Two residues indicated as changed in the lone example are residues 27 and 30 of the heavy chain (see Figure 1). Both are within a Chothia CDR. Queen '975 recites that the donor residue can be chosen if it falls into one or more of four different categories (see page 21, lines 19-34). Two residues indicated as changed in the lone example are residues 27 and 30 of the heavy chain (see Figure 1), both of which are within a Chothia CDR.</p>

wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	
and each of said donor amino acids: (I) is adjacent to a CDR in the donor immunoglobulin sequence or	
(II) contains an atom within a distance of 4 Å of a CDR in said humanized immunoglobulin.	No. Limitation not present in two earliest priority application. No distance is recited in Queen '975. Queen '252 recites a distance of "3 angstroms" (page 13, line 1). The distance has been extended in the claims as issued.

APPENDIX B

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1} .	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are so identified in Figure 4.

APPENDIX C

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4; page 7, lines 5-20.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 18, lines 11-17, and lines 33-37, and Figs. 20-21 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are identified in Figure 21.

Canonical Structures for the Hypervariable Regions of Immunoglobulins

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(Received 13 November 1986, and in revised form 23 April 1987)

We have analysed the atomic structures of Fab and V_L fragments of immunoglobulins to determine the relationship between their amino acid sequences and the three-dimensional structures of their antigen binding sites. We identify the relatively few residues that, through their packing, hydrogen bonding or the ability to assume unusual ϕ , ψ or ω conformations, are primarily responsible for the main-chain conformations of the hypervariable regions. These residues are found to occur at sites within the hypervariable regions and in the conserved β -sheet framework.

Examination of the sequences of immunoglobulins of unknown structure shows that many have hypervariable regions that are similar in size to one of the known structures and contain identical residues at the sites responsible for the observed conformation. This implies that these hypervariable regions have conformations close to those in the known structures. For five of the hypervariable regions, the repertoire of conformations appears to be limited to a relatively small number of discrete structural classes. We call the commonly occurring main-chain conformations of the hypervariable regions "canonical structures".

The accuracy of the analysis is being tested and refined by the prediction of immunoglobulin structures prior to their experimental determination.

1. Introduction

The specificity of immunoglobulins is determined by the sequence and size of the hypervariable regions in the variable domains. These regions produce a surface complementary to that of the antigen. The subject of this paper is the relation between the amino acid sequences of antibodies and the structure of their binding sites. The results we report are related to two previous sets of observations.

The first set concerns the sequences of the hypervariable regions. Kabat and his colleagues (Kabat *et al.*, 1977; Kabat, 1978) compared the sequences of the hypervariable regions then known and found that, at 13 sites in the light chains and at seven positions in the heavy chains, the residues are conserved. They argued that the residues at these sites are involved in the structure, rather than the specificity, of the hypervariable regions. They suggested that these residues have a fixed position in antibodies and that this could be used in the model building of combining sites to limit the conformations and positions of the sites whose residues varied. Padlan (1979) also examined the sequences of the hypervariable region of light

† Also associated with Fairleigh Dickinson University, Teaneck-Hackensack Campus, Teaneck, NJ 07666, U.S.A.

Table 2
Conformation of hair-pin turns

Structure	Sequence ^a	Conformation ^b (°)								Frequency ^c
	1 2 3 4 X·G·G·X	ϕ_2 +55	ψ_2 +35	ϕ_3 +85	ψ_3 -5 ^d					6/6
		or								
2 — 3 	X·G·X·X	+65	-125	-105	+10 ^e					6/7
1 — — 4 	X·X·G·X	+70	-115	-90	0 ^e					7/8
	X·X·X·X	+50	+45	+85	-20 ^e					4/4
	X·X·X·G	ϕ_1 -135	ψ_1 +175	ϕ_2 -50	ψ_2 -35	ϕ_3 -95	ψ_3 -10	ϕ_4 +145	ψ_4 +155	4/4
2 — 3 	1 2 3 4 5 ^f X X X X G	ϕ_2 -75	ψ_2 -10	ϕ_3 -95	ψ_3 -50	ϕ_4 -105	ψ_4 0	ϕ_5 +85	ψ_5 -160	3/3
1 — — 5 	X X X X X	+50	+55	+65	-50	-130	-5	-90	+130	1/1(3/3)
2 — 3 	1 2 3 4 5 ^f X·X·X·G·X	ϕ_2 -60	ψ_2 -25	ϕ_3 -90	ψ_3 0	ϕ_4 +85	ψ_4 +10			13/15
1 — — 5 	X·X·X·N·X D									
3 — 4 	1 2 3 4 5 6 ^g X·X·X·X·G·X	ϕ_2 -65	ψ_2 -30	ϕ_3 -65	ψ_3 -45	ϕ_4 -95	ψ_4 -5	ϕ_5 +70	ψ_5 +35	3/3
2 — 5 	X·X·X·X·N·X									2/2
1 — — 6 	X									1/1

The data in this Table are from an unpublished analysis of proteins whose atomic structure has been determined at a resolution of 2 Å or higher. The conformations described here for the 2-residue X·X·X·G turn and the 3-residue turns are new. The other conformations have been described by Sibanda & Thornton (1985) and by Efimov (1988). We list only conformations found more than once.

^a X indicates no residue restriction except that certain sites cannot have Pro, as this residue requires a ϕ value of $\sim -60^\circ$ and cannot form a hydrogen bond to its main-chain nitrogen.

^b Residues whose ϕ, ψ values are not given have a β conformation.

^c Frequencies are given as n_1/n_2 , where n_2 is the number of cases where we found the structure in column 1 with the sequence in column 2 and n_1 the number of these cases that have the conformation in column 3. Except for the frequencies in brackets, data is given only for non-homologous proteins.

^{d,e,f} These are type I', II' and III' turns.

^g Different conformations are found for the single cases of X·D·G·X·X and X·G·X·G·X.

^h Different conformations are found for the single cases of X·N·N·X·X, X·G·G·X·X and X·G·X·X·X.

ⁱ The 2 cases of X·X·X·X·X have different conformations.

^j Different conformations are found for the 2 cases of X·G·X·X·X·X.

these loops. This is discussed in sections 5 and 7, below.

The conservation of the framework structure extends to the residues immediately adjacent to the hypervariable regions. If the conserved frameworks of a pair of molecules are superposed, the differences in the positions of these residues is in most cases less than 1 Å and in all but one case less than 1.8 Å (Table 5). In contrast, residues in the hypervariable region adjacent to the conserved framework can differ in position by 3 Å or more.

The six loops, whose main-chain conformations vary and which are part of the antibody combining site, are formed by residues 26 to 32, 50 to 52 and 91 to 96 in V_L domains, and 28 to 32, 53 to 55 and 96 to 101 in the V_H domains L1, L2, L3, H1, H2 and H3, respectively. Their limits are somewhat different from those of the complementarity-determining regions defined by Kabat *et al.* (1983) on the basis of sequence variability: residues 24 to

34, 50 to 56 and 89 to 97 in V_L and 31 to 35, 50 to 65 and 95 to 102 in V_H . This point is discussed in section 11, below.

4. Conformation of the L1 Hypervariable Regions

In the known V_L structures, the conformations of the L1 regions, residues 26 to 32, are characteristic of the class of the light chain. In V_L domains their conformation is helical and in the V_H domains it is extended (Padlan *et al.*, 1977; Padlan, 1977b; de la Paz *et al.*, 1986). These conformational differences are the result of sequence differences in both the L1 region and the framework (Lesk & Chothia, 1982).

(a) V_L domains

Figure 4 shows the conformation of the L1 regions of the V_L domains. The L1 regions in RHE

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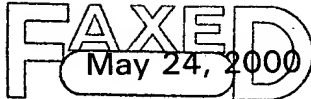
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11823-9

DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

CROSS-REFERENCE TO RELATED APPLICATION

5 This is a continuation-in-part application of commonly assigned patent application U.S.S.N. 290,975, filed December 28, 1988, which is incorporated herein by reference.

Field of the Invention

10 The present invention relates generally to the combination of recombinant DNA and monoclonal antibody technologies for developing novel therapeutic agents and, more particularly, to the production of non-immunogenic antibodies having strong affinity for a predetermined antigen.

15

Background of the Invention

20 The advent of monoclonal antibody technology in the mid 1970's heralded a new age of medicine. For the first time, researchers and clinicians had access to essentially unlimited quantities of uniform antibodies capable of binding to a predetermined antigenic site and having various immunological effector functions. These proteins, known as "monoclonal antibodies" were thought to hold great promise in, e.g., the removal of harmful cells in vivo. Indeed, the clinical value of monoclonal antibodies seemed limitless for this use alone.

25 Unfortunately, the development of appropriate therapeutic products based on these proteins has been severely hampered by a number of drawbacks inherent in monoclonal antibody production. For example, most monoclonal antibodies are mouse derived, and thus do not fix human complement well. They also lack other important immunoglobulin functional characteristics when used in humans.

35 Perhaps most importantly, non-human monoclonal antibodies contain substantial stretches of amino acid sequences that will be immunogenic when injected into a human

patient. Numerous studies have shown that after injection of a foreign antibody, the immune response mounted by a patient can be quite strong, essentially eliminating the antibody's therapeutic utility after an initial treatment. Moreover, as increasing numbers of different mouse or other antigenic (to humans) monoclonal antibodies can be expected to developed to treat various diseases, after the first or second treatments with any non-human antibodies, subsequent treatments, even for unrelated therapies, can be ineffective or even dangerous in themselves.

While the production of so called "chimeric antibodies" (e.g., mouse variable regions joined to human constant regions) has proven somewhat successful, a significant immunogenicity problem remains. Moreover, efforts to immortalize human B-cells or generate human hybridomas capable of producing human immunoglobulins against a desired antigen have been generally unsuccessful, particularly with many important human antigens. Most recently, recombinant DNA technology has been utilized to produce immunoglobulins which have human framework regions combined with complementarity determining regions (CDR's) from a donor mouse or rat immunoglobulin (see, e.g., EPO Publication No. 0239400, which is incorporated herein by reference). These new proteins are called "humanized immunoglobulins" and the process by which the donor immunoglobulin is converted into a human-like immunoglobulin by combining its CDR's with a human framework is called "humanization". Humanized antibodies are important because they bind to the same antigen as the original antibodies, but are less immunogenic when injected into humans.

However, a major problem with present humanization procedures has been a loss of affinity for the antigen, usually by at least 2 to 3-fold (Jones et al., Nature, 321:522-525 (1986)) and in some instances as much as 10-fold or more, especially when the antigen is a protein (Verhoeyen et al., Science, 239:1534-1536 (1988)). Loss of any affinity is, of course, highly undesirable. At the least, it means that more of the humanized antibody will have to be injected

into the patient, at higher cost and greater risk of adverse effects. Even more critically, an antibody with reduced affinity may have poorer biological functions, such as complement lysis, antibody-dependent cellular cytotoxicity, or virus neutralization. For example, the loss of affinity in the partially humanized antibody HuVHCAMP may have caused it to lose all ability to mediate complement lysis (see, Riechmann et al., Nature, 332:323-327 (1988); Table 1).

Thus, there is a need for improved means for producing humanized antibodies specifically reactive with strong affinity to a predetermined antigen. These humanized immunoglobulins should remain substantially non-immunogenic in humans, yet be easily and economically produced in a manner suitable for therapeutic formulation and other uses. The present invention fulfills these and other needs.

Summary of the Invention

The present invention provides novel methods for designing humanized immunoglobulin chains having one or more complementarity determining regions (CDR's) from a donor immunoglobulin and a framework region from a human immunoglobulin, the preferred methods comprising first comparing the framework or variable region amino acid sequence of the donor immunoglobulin to corresponding sequences in a collection of human immunoglobulin chains, and selecting as the human immunoglobulin one of the more homologous sequences from the collection. The human immunoglobulin, or acceptor immunoglobulin, sequence is typically selected from a collection of at least 10 to 20 immunoglobulin chain sequences, and usually will have the highest homology to the donor immunoglobulin sequence of any sequence in the collection. The human immunoglobulin framework sequence will typically have about 65 to 70% homology or more to the donor immunoglobulin framework sequences. The donor immunoglobulin may be either a heavy chain or light chain (or both), and the human collection will contain the same kind of chain. A humanized light and heavy chain can be used to form a complete humanized immunoglobulin

or antibody, having two light/heavy chain pairs, with or without partial or full-length human constant regions and other proteins.

In another embodiment of the present invention, either in conjunction with the above comparison step or separately, additional amino acids in an acceptor immunoglobulin chain may be replaced with amino acids from the CDR-donor immunoglobulin chain. More specifically, further optional substitutions of a human framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from a donor immunoglobulin will be made at positions in the immunoglobulins where:

(a) the amino acid in the human framework region of an acceptor immunoglobulin is rare for that position and the corresponding amino acid in the donor immunoglobulin is common for that position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to be within about 3Å of the CDR's in a three-dimensional immunoglobulin model and capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.

The humanized immunoglobulin chain will typically comprise at least about 3 amino acids from the donor immunoglobulin in addition to the CDR's, usually at least one of which is immediately adjacent to a CDR in the donor immunoglobulin. The heavy and light chains may each be designed by using any one or all three of the position criteria.

When combined into an intact antibody, the humanized light and heavy chains of the present invention will be substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen (such as a protein or other compound containing an epitope). These affinity levels can vary from about 10^8 M^{-1} or higher, and may be within about 4 fold of the donor immunoglobulin's original affinity to the antigen.

Once designed, the immunoglobulins, including binding fragments and other immunoglobulin forms, of the present invention may be produced readily by a variety of recombinant DNA or other techniques. Preferably, polynucleotides encoding the desired amino acid sequences are produced synthetically or by joining appropriate nucleic acid sequences for expression in a suitable host (e.g., cell culture). The humanized immunoglobulins will be particularly useful in treating human disorders susceptible to monoclonal antibody therapy, but find a variety of other uses as well.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Comparison of sequences of anti-Tac heavy chain (upper lines) and Eu heavy chain (lower lines). The 1-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDR's are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an *.

Figure 2. Comparison of sequences of anti-Tac light chain (upper lines) and Eu light chain (lower lines). The single-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDR's are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an *.

Figure 3. Nucleotide sequence of the gene for the humanized anti-Tac heavy chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature heavy chain sequence begins with amino acid #20 Q.

Figure 4. Nucleotide sequence of the gene for the humanized anti-Tac light chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature light chain sequence begins with amino acid #21 D.

Figure 5. A. Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac heavy chain gene, printed 5' to 3'. B. Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide.

Figure 6. (A) Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac light chain gene, printed 5' to 3'. (B) Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide. The position of a Hind III site in the overlap of JFD2 and JFD3 is shown.

Figure 7. Schematic diagram of the plasmid pHuGTAC1 used to express the humanized anti-Tac heavy chain. Relevant restriction sites are shown, and coding regions of the heavy chain are displayed as boxes. The direction of transcription from the immunoglobulin (Ig) promoter is shown by an arrow. E_H = heavy chain enhancer, Hyg = hygromycin resistance gene.

Figure 8. Schematic diagram of the plasmid pHuLTAC used to express the humanized anti-Tac light chain. Relevant restriction sites are shown, and coding regions of the light chain are displayed as boxes. The direction of transcription from the Ig promoter is shown by an arrow.

Figure 9. Fluorocytometry of HUT-102 and Jurkat cells stained with anti-Tac antibody or humanized anti-Tac antibody followed respectively by fluorescein-conjugated goat anti-mouse Ig antibody or goat anti-human Ig antibody, as labeled. In each panel, the dotted curve shows the results when the first antibody was omitted, and the solid curve the results when first and second (conjugated) antibodies were included as described.

Figure 10. (A) Fluorocytometry of HUT-102 cells stained with 0-40 ng of anti-Tac as indicated, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin. (B) Fluorocytometry of HUT-102 cells stained with the indicated antibody, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, novel means of designing humanized immunoglobulins capable of specifically binding to a predetermined antigen with strong affinity are provided. These improved methods produce immunoglobulins that are substantially non-immunogenic in humans but have binding affinities of at least about 10^8 M^{-1} , preferably 10^9 M^{-1} to 10^{10} M^{-1} , or stronger. The humanized immunoglobulins will have a human framework and have one or more complementary determining regions (CDR's), plus a limited number of other amino acids, from a donor immunoglobulin specifically reactive with an antigen. The immunoglobulins can be produced economically in large quantities and find use, for example, in the treatment of various human disorders by a variety of techniques.

In order that the invention may be more completely understood, several definitions are set forth. As used herein, the term "immunoglobulin" refers to a protein having one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 Kd, about 214 amino acids) are encoded by a variable region gene at the NH₂-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH - terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd, about 446 amino acids), are similarly encoded by a variable region gene (encoding about 116 amino acids) and one of the other

aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

One form of immunoglobulin constitutes the basic structural unit of an antibody. This form is a tetramer and consists of two identical pairs of immunoglobulin chains, each pair having one light and one heavy chain. In each pair, the light and heavy chain variable regions are together responsible for binding to an antigen, and the constant regions are responsible for the antibody effector functions. In addition to antibodies, immunoglobulins may exist in a variety of other forms (including less than full-length that retain the desired activities), including, for example, Fv, Fab, and F(ab')₂, as well as single chain antibodies (e.g., Huston et al., Proc. Nat. Acad. Sci. U.S.A., 85:5879-5883 (1988) and Bird et al., Science, 242:423-426 (1988), which are incorporated herein by reference). (See, generally, Hood et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, Nature, 323:15-16 (1986), which are incorporated herein by reference).

An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, also called CDR's. The extent of the framework region and CDR's have been precisely defined (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services, (1983); which is incorporated herein by reference). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDR's. The CDR's are primarily responsible for binding to an epitope of an antigen.

Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant region genes belonging to different species. For example, the variable segments of the genes from a mouse monoclonal antibody may be joined to human constant segments, such as

gamma 1 and gamma 3. A typical therapeutic chimeric antibody is thus a hybrid protein composed of the variable or antigen-binding domain from a mouse antibody and the constant or effector domain from a human antibody (e.g., A.T.C.C. Accession No. CRL 9688 secretes an anti-Tac chimeric antibody), although other mammalian species may be used.

As used herein, the term "humanized" immunoglobulin refers to an immunoglobulin comprising a substantially human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the "donor" and a human immunoglobulin providing the framework is called the "acceptor". Constant regions need not be present, but if they are, they must be substantially homologous to human immunoglobulin constant regions, i.e., at least about 85-90%, preferably about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's, are substantially homologous to corresponding parts of natural human immunoglobulin sequences. A "humanized antibody" is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. For example, a humanized antibody would not encompass a typical chimeric antibody as defined above, e.g., because the entire variable region of a chimeric antibody is non-human. One says that the donor antibody has been "humanized", by the process of "humanization", because the resultant humanized antibody is expected to bind to the same antigen as the donor antibody that provides the CDR's.

Humanized immunoglobulins, including humanized antibodies, have been constructed by means of genetic engineering. Most humanized immunoglobulins that have been previously described (Jones et al., op. cit.; Verhoeven et al., op. cit.; Riechmann et al., op. cit.) have comprised a framework that is identical to the framework of a particular human immunoglobulin chain, the acceptor, and three CDR's from a non-human donor immunoglobulin chain. In one case (Riechmann et al., op. cit.), two additional amino acids in the framework were changed to be the same as amino acids in

other human framework regions. The present invention includes criteria by which a limited number of amino acids in the framework of a humanized immunoglobulin chain are chosen to be the same as the amino acids at those positions in the donor rather than in the acceptor, in order to increase the affinity of an antibody comprising the humanized immunoglobulin chain.

The present invention is based in part on the model that two contributing causes of the loss of affinity in prior means of producing humanized antibodies (using as examples mouse antibodies as the source of CDR's) are:

(1) When the mouse CDR's are combined with the human framework, the amino acids in the framework close to the CDR's become human instead of mouse. Without intending to be bound by theory, we believe that these changed amino acids may slightly distort the CDR's, because they create different electrostatic or hydrophobic forces than in the donor mouse antibody, and the distorted CDR's may not make as effective contacts with the antigen as the CDR's did in the donor antibody;

(2) Also, amino acids in the original mouse antibody that are close to, but not part of, the CDR's (i.e., still part of the framework), may make contacts with the antigen that contribute to affinity. These amino acids are lost when the antibody is humanized, because all framework amino acids are made human.

To avoid these problems, and to produce humanized antibodies that have a very strong affinity for a desired antigen, the present invention uses the following four criteria for designing humanized immunoglobulins. These criteria may be used singly, or when necessary in combination, to achieve the desired affinity or other characteristics.

Criterion I: As acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. For example,

comparison of the sequence of a mouse heavy (or light) chain variable region against human heavy (or light) variable regions in a data bank (for example, the National Biomedical Research Foundation Protein Identification Resource) shows that the extent of homology to different human regions varies greatly, typically from about 40% to about 60-70%. By choosing as the acceptor immunoglobulin one of the human heavy (respectively light) chain variable regions that is most homologous to the heavy (respectively light) chain variable region of the donor immunoglobulin, fewer amino acids will be changed in going from the donor immunoglobulin to the humanized immunoglobulin. Hence, and again without intending to be bound by theory, it is believed that there is a smaller chance of changing an amino acid near the CDR's that distorts their conformation. Moreover, the precise overall shape of a humanized antibody comprising the humanized immunoglobulin chain may more closely resemble the shape of the donor antibody, also reducing the chance of distorting the CDR's.

Typically, one of the 3-5 most homologous heavy chain variable region sequences in a representative collection of at least about 10 to 20 distinct human heavy chains will be chosen as acceptor to provide the heavy chain framework, and similarly for the light chain. Preferably, one of the 1-3 most homologous variable regions will be used. The selected acceptor immunoglobulin chain will most preferably have at least about 65% homology in the framework region to the donor immunoglobulin.

Regardless of how the acceptor immunoglobulin is chosen, higher affinity may be achieved by selecting a small number of amino acids in the framework of the humanized immunoglobulin chain to be the same as the amino acids at those positions in the donor rather than in the acceptor. The following criteria define what amino acids may be so selected. Preferably, at most or all amino acid positions satisfying one of these criteria, the donor amino acid will in fact be selected.

Criterion II: If an amino acid in the framework of the human acceptor immunoglobulin is unusual (*i.e.*, "rare", which as used herein indicates an amino acid occurring at that position in no more than about 10% of human heavy (respectively light) chain V region sequences in a representative data bank), and if the donor amino acid at that position is typical for human sequences (*i.e.*, "common", which as used herein indicates an amino acid occurring in at least about 25% of sequences in a representative data bank), then the donor amino acid rather than the acceptor may be selected. This criterion helps ensure that an atypical amino acid in the human framework does not disrupt the antibody structure. Moreover, by replacing an unusual amino acid with an amino acid from the donor antibody that happens to be typical for human antibodies, the humanized antibody may be made less immunogenic.

Criterion III: In the positions immediately adjacent to the 3 CDR's in the humanized immunoglobulin chain, the donor amino acid rather than acceptor amino acid may be selected. These amino acids are particularly likely to interact with the amino acids in the CDR's and, if chosen from the acceptor, distort the donor CDR's and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit *et al.*, *Science*, 233, 747-753 (1986), which is incorporated herein by reference) and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

Criterion IV: A 3-dimensional model, typically of the original donor antibody, shows that certain amino acids outside of the CDR's are close to the CDR's and have a good probability of interacting with amino acids in the CDR's by hydrogen bonding, Van der Waals forces, hydrophobic interactions, etc. At those amino acid positions, the donor amino acid rather than the acceptor immunoglobulin amino acid may be selected. Amino acids according to this criterion will

generally have a side chain atom within about 3 angstrom units of some site in the CDR's and must contain atoms that could interact with the CDR atoms according to established chemical forces, such as those listed above. Computer programs to create models of proteins such as antibodies are generally available and well known to those skilled in the art (see, Loew et al., Int. J. Quant. Chem., Quant. Biol. Symp., 15:55-66 (1988); Bruccoleri et al., Nature, 335, 564-568 (1988); Chothia et al., Science, 233:755-758 (1986), all of which are incorporated herein by reference). These do not form part of the invention. Indeed, because all antibodies have similar structures, the known antibody structures, which are available from the Brookhaven Protein Data Bank, can be used if necessary as rough models of other antibodies. Commercially available computer programs can be used to display these models on a computer monitor, to calculate the distance between atoms, and to estimate the likelihood of different amino acids interacting (see, Ferrin et al., J. Mol. Graphics, 6:13-27 (1988).)

Humanized antibodies generally have at least three potential advantages over mouse or in some cases chimeric antibodies for use in human therapy:

1) Because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).

2) The human immune system should not recognize the framework or constant region of the humanized antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign mouse antibody or a partially foreign chimeric antibody.

3) Injected mouse antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of normal antibodies (D. Shaw et al., J. Immunol., 138:4534-4538 (1987).) Injected humanized antibodies will presumably have a half-life more similar to

naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

In one aspect, the present invention is directed to designing humanized immunoglobulins that are produced by expressing recombinant DNA segments encoding the heavy and light chain CDR's from a donor immunoglobulin capable of binding to a desired antigen, such as the human IL-2 receptor, to DNA segments encoding acceptor human framework regions. Exemplary DNA sequences designed in accordance with the present invention and, which on expression code for the polypeptide chains comprising heavy and light chain CDR's with substantially human framework regions, are shown in Figures 3 and 4, respectively. Due to codon degeneracy and non-critical amino acid substitutions, other DNA sequences can be readily substituted for those sequences, as detailed below. In general, the criteria of the present invention find applicability to designing substantially any humanized immunoglobulin.

The DNA segments will typically further include an expression control DNA sequence operably linked to the humanized immunoglobulin coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the humanized light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow (see, S. Beychok, Cells of Immunoglobulin Synthesis, Academic Press, N.Y., (1979), which is incorporated herein by reference.

Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B-cells (see, Kabat

op. cit. and WP87/02671). The CDR's for producing the immunoglobulins of the present invention will be similarly derived from monoclonal antibodies capable of binding to the predetermined antigen, such as the human IL-2 receptor, and produced by well known methods in any convenient mammalian source including, mice, rats, rabbits, or other vertebrate capable of producing antibodies. Suitable source cells for the constant region and framework DNA sequences, and host cells for immunoglobulin expression and secretion, can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference).

In addition to the humanized immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins to the native sequences can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary specifically from the sequences in Figures 3 and 4 at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Gillman and Smith, Gene, 8:81-97 (1979) and S. Roberts et al., Nature, 328:731-734 (1987), both of which are incorporated herein by reference).

Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). Also because like many genes, the immunoglobulin-related genes contain separate functional regions, each having one or more distinct biological activities, the genes may be fused to functional regions from other genes (e.g., enzymes, see,

commonly assigned U.S.S.N. 132,387, filed Dec. 15, 1987, which is incorporated herein by reference) to produce fusion proteins (e.g., immunotoxins) having novel properties. The nucleic acid sequences of the present invention capable of ultimately expressing the desired humanized antibodies can be
5 formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), as well as by a variety of different techniques. Joining appropriate genomic sequences is presently the most common method of production,
10 but cDNA sequences may also be utilized (see, European Patent Publication No. 0239400 and L. Reichmann et al., Nature, 332:323-327 (1988), both of which are incorporated herein by reference).

As stated previously, the DNA sequences will be
15 expressed in hosts after the sequences have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly,
20 expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences (see, e.g., U.S. Patent 4,704,362, which is incorporated herein by reference).

E. coli is one prokaryotic host useful particularly
25 for cloning the DNA sequences of the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression
30 vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (*trp*) promoter system, a beta-lactamase
35 promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site

sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may also be used for expression. *Saccharomyces* is a preferred host, with suitable vectors having expression control sequences, such as
5 promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the
10 polypeptides of the present invention (see, Winnacker, "From Genes to Clones," VCH Publishers, N.Y., N.Y. (1987), which is incorporated herein by reference). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been
15 developed in the art, and include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, etc, but preferably transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an
20 enhancer (Queen et al., Immunol. Rev., 89:49-68 (1986), which is incorporated herein by reference), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences
25 are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, and the like.

The vectors containing the DNA segments of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the
30 host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts. (See, generally, Maniatis et al.,
35 Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, (1982), which is incorporated herein by reference.)

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, R. Scopes, "Protein Purification", Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures, immunofluorescent stainings, and the like. (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The antibodies of the present invention will typically find use individually in treating substantially any disease susceptible to monoclonal antibody-based therapy. In particular, the immunoglobulins can be used for passive immunization or the removal of unwanted cells or antigens, such as by complement mediated lysis, all without substantial immune reactions (e.g., anaphylactic shock) associated with many prior antibodies. For example, where the cell linked to a disease has been identified as IL-2 receptor bearing, then humanized antibodies that bind to the human IL-2 receptor are suitable (see, U.S.S.N. 085,707, entitled "Treating Human Malignancies and Disorders," which is incorporated herein by reference). For such a humanized immunoglobulin, typical disease states suitable for treatment include graft versus host disease and transplant rejection in patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc. Other diseases include autoimmune diseases, such as Type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis.

The method of producing humanized antibodies of the present invention can be used to humanize a variety of donor

antibodies, especially monoclonal antibodies reactive with markers on cells responsible for a disease. For example, suitable antibodies bind to antigens on T-cells, such as those grouped into the so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Bernard et al., Eds., Springer-Verlag, N.Y. (1984), which is incorporated herein by reference.

The antibodies of the present invention can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

A preferred pharmaceutical composition of the present invention comprises the use of the subject antibodies in immunotoxins. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle," provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al., Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), which is incorporated herein by reference.

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides,

such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). (See, generally, commonly assigned U.S.S.N. 290,968 (Townsend and Townsend Docket No. 11823-7-2) filed in U.S.P.T.O. on December 28, 1988, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985), all of which are incorporated herein by reference.)

The delivery component of the immunotoxin will include the humanized immunoglobulins of the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

For diagnostic purposes, the antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the humanized antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme co-factors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

The following examples are offered by way of illustration, not by limitation.

EXPERIMENTAL

Design of genes for humanized light and heavy chains

The sequence of the human antibody Eu (Sequences of Proteins of Immunological Interest, E. Kabat et al., U.S. Dept. of Health and Human Services, 1983) was used to provide the framework of the humanized antibody, because the amino acid sequence of the heavy chain variable region of anti-Tac is more homologous to the heavy chain of this antibody than to any other complete heavy chain variable region sequence in the National Biomedical Foundation Protein Identification Resource.

To select the sequence of the humanized heavy chain, the anti-Tac heavy chain sequence (see, commonly assigned U.S.S.N.'s 186,862 and 223,037, which are incorporated herein by reference) was aligned with the sequence of the Eu heavy chain (Figure 1). At each position, the Eu amino acid was selected for the humanized sequence, unless that position fell in any one of the following categories, in which case the anti-Tac amino acid was selected:

(1) The position fell within a complementarity determining region (CDR), as defined by Kabat, et al., op. cit. (amino acids 31-35, 50-66, 99-106);

(2) The Eu amino acid was rare for human heavy chains at that position, whereas the anti-Tac amino acid was common for human heavy chains at that position (amino acids 27, 93, 95, 98, 107-109, 111);

(3) The position was immediately adjacent to a CDR in the amino acid sequence of the anti-Tac heavy chain (amino acids 30 and 67); or

(4) 3-dimensional modeling of the anti-Tac antibody suggested that the amino acid was physically close to the antigen binding region (amino acids 48 and 68).

Amino acid #27 is listed in category (4) because the acceptor Eu amino acid Gly is rare, and the donor anti-Tac amino acid Tyr is chemically similar to the amino acid Phe, which is common, but the substitution was actually made because #27

also fell in category (4). Although some amino acids fell in more than one of these categories, they are only listed in one. Categories (2) - (4) correspond to criteria (2) - (4) described above.

To select the sequence of the humanized light chain, the anti-Tac light chain sequence was aligned with the sequence of the Eu light chain (Figure 2). The Eu amino acid was selected at each position, unless the position again fell into one of the categories (1) - (4) (with light chain replacing heavy chain in the category definitions):

- (1) CDR's (amino acids 24-34, 50-56, 89-97);
- (2) Anti-Tac amino acid more typical than Eu (amino acids 48 and 63);
- (3) Adjacent to CDR's (no amino acids; Eu and anti-Tac were already the same at all these positions); or
- (4) Possible 3-dimensional proximity to binding region (amino acid 60).

The actual nucleotide sequence of the heavy (Figure 3) and light chain (Figure 4) genes were selected as follows:

- (1) The nucleotide sequences code for the amino acid sequences chosen as described above;
- (2) 5' of these coding sequences, the nucleotide sequences code for a leader (signal) sequence, namely the leader of the light chain of the antibody MOPC 63 and the leader of the heavy chain of the antibody PCH 108A (Kabat et al., op. cit.). These leader sequences were chosen as typical of antibodies;
- (3) 3' of the coding sequences, the nucleotide sequences are the sequences that follow the mouse light chain J5 segment and the mouse heavy chain J2 segment, which are part of the anti-Tac sequences. These sequences are included because they contain splice donor signals; and
- (4) At each end of the sequence is an Xba I site to allow cutting at the Xba I sites and cloning into the Xba I site of a vector.

Construction of humanized light and heavy chain genes

To synthesize the heavy chain, four oligonucleotides HES12, HES13, HES14, HES15 (Figure 5A) were synthesized using an Applied Biosystems 380B DNA synthesizer. Two of the oligonucleotides are part of each strand of the heavy chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 5B). Together, the oligonucleotides cover the entire humanized heavy chain variable region (Figure 3) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

Each oligonucleotide was phosphorylated using ATP and T4 polynucleotide kinase by standard procedures (see, Maniatis, op. cit.). To anneal the phosphorylated oligonucleotides, they were suspended together in 40 ul of TA (33 mM Tris acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate) at a concentration of about 3.75 uM each, heated to 95 deg for 4 min. and cooled slowly to 4 deg. To synthesize the complete gene from the oligonucleotides by synthesizing the opposite strand of each oligonucleotide (Figure 5B), the following components were added in a final volume of 100ul:

	10 ul	annealed oligonucleotides
	0.16 mM each	deoxyribonucleotide
25	0.5 mM	ATP
	0.5 mM	DTT
	100 ug/ml	BSA
	3.5 ug/ml	T4 g43 protein (DNA polymerase)
30	25 ug/ml	T4 g44/62 protein (polymerase accessory protein)
	25 ug/ml	45 protein (polymerase accessory protein)

The mixture was incubated at 37 deg for 30 min. Then 10 u of T4 DNA ligase was added and incubation at 37 deg resumed for 30 min. The polymerase and ligase were inactivated by incubation of the reaction at 70 deg for

15 min. To digest the gene with Xba I, to the reaction was added 50 ul of 2x TA containing BSA at 200 ug/ml and DTT at 1 mM, 43 ul of water, and 50 u of Xba I in 5 ul. The reaction was incubated for 3 hr at 37 deg, and run on a gel. The 431 bp Xba I fragment was purified from a gel and cloned into the Xba I site of the plasmid pUC19 by standard methods. Four plasmid isolates were purified and sequenced using the dideoxy method. One of these had the correct sequence (Figure 3).

To synthesize the light chain, four oligonucleotides JFD1, JFD2, JFD3, JFD4 (Figure 6A) were synthesized. Two of the oligonucleotides are part of each strand of the light chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 6B). Together, the oligonucleotides cover the entire humanized light chain variable region (Figure 4) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

The light chain gene was synthesized from these oligonucleotides in two parts. 0.5 ug each of JFD1 and JFD2 were combined in 20 ul sequenase buffer (40 mM Tris-HCl, pH 7.5, 20 mM magnesium chloride, 50 mM sodium chloride), heated at 70 deg for 3 min and allowed to cool slowly to 23 deg in order for the oligonucleotides to anneal. JFD3 and JFD4 were treated in the same way. Each reaction was made 10 mM in DTT and 0.5 mM in each deoxyribonucleotide and 6.5 u of sequenase (US Biochemicals) was added, in a final volume of 24 ul, and incubated for 1 hr at 37 deg to synthesize the opposite strands of the oligonucleotides. Xba I and Hind III were added to each reaction to digest the DNA (there is a Hind III site in the region where JFD2 and JFD3 overlap and therefore in each of the synthesized DNAs; Figure 6B). The reactions were run on polyacrylamide gels, and the Xba I - Hind III fragments were purified and cloned into pUC18 by standard methods. Several plasmid isolates for each fragment were sequenced by the dideoxy method, and correct ones chosen.

Construction of plasmids to express humanized light and heavy chains

The heavy chain Xba I fragment was isolated from the pUC19 plasmid in which it had been inserted and then inserted into the Xba I site of the vector pVγ1 (see, commonly assigned U.S.S.N. 223,037) in the correct orientation by standard methods, to produce the plasmid pHuGTAC1 (Figure 7). This plasmid will express high levels of a complete heavy chain when transfected into an appropriate host cell.

The two light chain Xba I - Hind III fragments were isolated from the pUC18 plasmids in which they had been inserted. The vector plasmid pVκ1 (see, commonly assigned U.S.S.N. 223,037) was cut with Xba I, dephosphorylated and ligated with the two fragments by standard methods. The desired reaction product has the circular form: vector - Xba I - fragment 1 - Hind III - fragment 2 - Xba I - vector. Several plasmid isolates were analyzed by restriction mapping and sequencing, and one with this form chosen. This plasmid, pHuLTAC (Figure 8), therefore contains the complete humanized light chain (Figure 4) and will express high levels of the light chain when transfected into an appropriate host cell.

Synthesis and affinity of humanized antibody

The plasmids pHuGTAC1 and pHuLTAC were transfected into mouse Sp2/0 cells, and cells that integrated the plasmids were selected on the basis of resistance to mycophenolic acid and/or hygromycin B conferred by the gpt and hyg genes on the plasmids (Figures 7,8) by standard methods. To verify that these cells secreted antibody that binds to the IL-2 receptor, supernatant from the cells was incubated with HUT-102 cells that are known to express the IL-2 receptor. After washing, the cells were incubated with fluorescein-conjugated goat anti-human antibody, washed, and analyzed for fluorescence on a FACSCAN cytofluorometer. The results (Figure 9A), clearly show that the humanized antibody binds to these cells, but not to Jurkat T-cells that do not express the IL-2 receptor (Figure 9D). As controls, the

original mouse anti-Tac antibody was also used to stain these cells (Figure 9B,C), giving similar results.

For further experiments, cells producing the humanized antibody were injected into mice, and the resultant ascites collected. Humanized antibody was purified to
5 substantial homogeneity from the ascites by passage through an affinity column of goat anti-human immunoglobulin antibody, prepared on an Affigel-10 support (Bio-Rad Laboratories, Inc., Richmond, CA) according to standard techniques. To determine the affinity of the humanized
10 antibody relative to the original anti-Tac antibody, a competitive binding experiment was performed. About 5×10^5 HUT-102 cells were incubated with known quantities (10 - 40 ng) of the anti-Tac antibody and the humanized anti-Tac antibody for 10 min at 4 deg. Then 100 ng of biotinylated
15 anti-Tac was added to the cells and incubated for 30 min at 4 deg. This quantity of anti-Tac had previously been determined to be sufficient to saturate the binding sites on the cells, but not to be in large excess. Then the cells were washed twice with 2 ml of phosphate buffered saline
20 (PBS) containing 0.1% sodium azide. The cells were then incubated for 30 min at 4 deg with 250 ng of phycoerythrin-conjugated avidin, which bound to the biotinylated anti-Tac already bound to the cells. The cells were washed again as above, fixed in PBS containing 1%
25 paraformaldehyde, and analyzed for fluorescence on a FACSCAN cytofluorometer.

Use of increasing amounts (10 - 40 ng) of the anti-Tac antibody as competitor in the first step decreased the amount of biotinylated anti-Tac that could bind to the
30 cells in the second step, and therefore the amount of phycoerythrin-conjugated avidin that bound in the last step, thus decreasing fluorescence (Figure 10A). Equivalent amounts (20 ng) of anti-Tac, and humanized anti-Tac used as competitor decreased the fluorescence to approximately the
35 same degree (Figure 10B). This shows that these antibodies have approximately the same affinity, because if one had greater affinity, it would have more effectively competed

with the biotinylated anti-Tac, thus decreasing fluorescence more.

Biological properties of the humanized antibody

For optimal use in treatment of human disease, the humanized antibody should be able to destroy T-cells in the body that express the IL-2 receptor. One mechanism by which antibodies may destroy target cells is antibody-dependent cell-mediated cytotoxicity, abbreviated ADCC (Fundamental Immunology, Paul, W., Ed., Raven Press, New York (1984), at pg. 681), in which the antibody forms a bridge between the target cell and an effector cell such as a macrophage that can lyse the target. To determine whether the humanized antibody and the original mouse anti-Tac antibody can mediate ADCC, a chromium release assay was performed by standard methods. Specifically, human leukemia HUT-102 cells, which express the IL-2 receptor, were incubated with ^{51}Cr to allow them to absorb this radionuclide. The HUT-102 cells were then incubated with an excess of either anti-Tac or humanized anti-Tac antibody. The HUT-102 cells were next incubated for 4 hrs with either a 30:1 or 100:1 ratio of effector cells, which were normal purified human peripheral blood mononuclear cells that had been activated by incubation for about 20 hrs with human recombinant IL-2. Release of ^{51}Cr , which indicated lysis of the target HUT-102 cells, was measured and the background subtracted (Table 1). The results show that at either ratio of effector cells, anti-Tac did not lyse a significant number of the target cells (less than 5%), while the humanized antibody did (more than 20%). Hence, the humanized antibody is likely to be more efficacious than the original mouse antibody in treating T-cell leukemia or other T-cell mediated diseases.

TABLE 1

5 Percent ^{51}Cr release after ADCC

Effector: Target ratio
30:1 100:1

10 Antibody

Anti-Tac	4%	< 1%
Humanized anti-Tac	24%	23%

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Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

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WE CLAIM:

- Sub A1*
1. A method of designing a humanized immunoglobulin (Ig) chain having one or more complementarity determining regions (CDR's) from a donor Ig and a framework region from a human Ig, said method comprising: comparing the framework or variable region amino acid sequence of the donor Ig with corresponding sequences in a collection of human Ig chains; and selecting to provide the human Ig framework one of the about three most homologous sequences from the collection.
 2. A method according to Claim 1, wherein the human Ig sequence is selected from a collection of at least about ten to twenty Ig chain sequences.
 3. A method according to Claim 1, wherein the human Ig chain sequence selected has the highest homology in the collection to the donor Ig sequence.
 4. A method according to Claim 1, wherein the human Ig framework sequence selected is at least about 65% homologous to the donor Ig framework sequence.
 5. A method according to Claim 1, wherein the immunoglobulin chain is a heavy chain.
 6. A method according to Claim 1, wherein the humanized Ig chain comprises a human constant region.
 7. An immunoglobulin comprising two light/heavy chain pairs, wherein at least one chain is designed in accordance with Claim 1.

8. A method of designing a humanized immunoglobulin chain having a framework region from a human acceptor immunoglobulin and complementarity determining regions (CDR's) from a donor immunoglobulin capable of binding to an antigen, said method comprising the steps of substituting at least one human framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from the donor immunoglobulin at a position in the immunoglobulins where:

(a) the amino acid in the human framework region of the acceptor immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to have a side chain atom within about 3Å of the CDR's in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.

9. A method according to Claim 8, wherein the humanized immunoglobulin chain comprises in addition to the CDR's at least three amino acids from the donor immunoglobulin chosen by criteria (a), (b) or (c).

10. A method according to Claim 9, wherein at least one of the amino acids substituted from the donor is immediately adjacent a CDR.

11. A method according to Claim 9, wherein said humanized immunoglobulin chain is a heavy chain.

12. An immunoglobulin comprising two light/heavy chain pairs, wherein at least one chain is designed in accordance with Claim 8.

13. An immunoglobulin according to Claim 12, which is specifically reactive with an antigen at an affinity of at least about 10^8 M^{-1} or stronger.

14. An immunoglobulin according to Claim 12, wherein the designed chain is a light chain comprising about 214 amino acids.

15. An immunoglobulin according to Claim 12, wherein the designed chain is a heavy chain comprising about 446 amino acids.

16. A DNA sequence which upon expression encodes a humanized immunoglobulin chain according to Claim 1 or Claim 8.

17. A method for improving the affinity of a humanized immunoglobulin (Ig) to an antigen, by replacing amino acids of the human Ig framework with amino acids from the donor Ig framework at positions where:

(a) the amino acid in the human framework region of the first immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to have a side chain atom within about 3Å of the CDR's in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or the CDR's of the humanized immunoglobulin.

18. A method according to Claim 17, wherein the additional amino acids comprise up to three amino acids, each of which is immediately adjacent to one of the CDR's in the second Ig.

19. A method according to Claim 17, wherein the additional amino acids comprise one amino acid immediately adjacent to a CDR.

5 20. A method according to Claim 17, wherein the additional amino acids comprise at least two amino acids from the donor Ig which are predicted by modelling to be capable of interacting with the antigen or the CDR's.

10 21. A method according to Claim 20, wherein said two or more amino acids are predicted to be within about 3A of the donor Ig CDR's.

15 22. A method according to Claim 17, wherein the humanized Ig has an affinity to the antigen within about 2 to 3 fold of the donor Ig.

23. A method according to Claim 17, wherein the antigen is a protein.

20 24. A method of producing a humanized immunoglobulin containing a heavy chain and a light chain designed in accordance with Claim 17, said method comprising:
25 culturing a host capable of expressing said heavy chain, said light chain, or both, under conditions suitable for production of said chains; and
recovering from the culture said humanized immunoglobulin.

30 25. A polynucleotide composition comprising a DNA sequence coding for a humanized immunoglobulin designed in accordance with Claim 17.

35 26. A method of producing an improved humanized immunoglobulin comprising expressing the polynucleotide composition of Claim 25.

27. A cell transformed with a polynucleotide composition according to Claim 25.

28. A composition comprising a humanized immunoglobulin secreted by a cell line according to Claim 24.

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DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

ABSTRACT OF THE DISCLOSURE

5 Novel methods for designing humanized
immunoglobulins having one or more complementarity
determining regions (CDR's) from a donor immunoglobulin and a
framework region from a human immunoglobulin comprising first
10 comparing the framework or variable region amino acid
sequence of the donor immunoglobulin to corresponding
- sequences in a collection of human immunoglobulin chains, and
selecting as the human immunoglobulin one of the more
homologous sequences from the collection. Each humanized
immunoglobulin chain may comprise about 3 or more amino acids
15 from the donor immunoglobulin in addition to the CDR's,
usually at least one of which is immediately adjacent to a
CDR in the donor immunoglobulin. The heavy and light chains
may each be designed by using any one or all three additional
20 position criteria. When combined into an intact antibody,
the humanized immunoglobulins of the present invention will
be substantially non-immunogenic in humans and retain
substantially the same affinity as the donor immunoglobulin
to the antigen, such as a protein or other compound
containing an epitope.

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WP50/ PDL/ PA9.PTO

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FIGURE 1

1	Q	V	Q	L	Q	Q	S	G	A	E	L	A	K	P	G	A	S	V	K	M
1	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V
21	S	C	K	A	S	G	Y	T	F	T	S	Y	R	M	H	W	V	K	Q	R
21	S	C	K	A	S	G	G	T	F	S	R	S	A	I	I	W	V	R	Q	A
							*			*	-----									
41	P	G	Q	G	L	E	W	I	G	Y	I	N	P	S	T	G	Y	T	E	Y
41	P	G	Q	G	L	E	W	M	G	G	I	V	P	M	F	G	P	P	N	Y
								*	-----											
61	N	Q	K	F	K	D	K	A	T	L	T	A	D	K	S	S	S	T	A	Y
61	A	Q	K	F	Q	G	R	V	T	I	T	A	D	E	S	T	N	T	A	Y
	-----						*	*												
81	M	Q	L	S	S	L	T	F	E	D	S	A	V	Y	Y	C	A	R	G	
81	M	E	L	S	S	L	R	S	E	D	T	A	F	Y	F	C	A	G	G	Y
													*		*			*	-----	
100	G	G	V	F	D	Y	W	G	Q	G	T	T	L	T	V	S	S			
101	G	I	Y	S	P	E	E	Y	N	G	G	L	V	T	V	S	S			
	-----						*	*	*	*										

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1		Q	I	V	L	T	Q	S	P	A	I	M	S	A	S	P	G	E	K	V	T
1		D	I	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	T
21		I	T	C	S	A	S	S	S	I		S	Y	M	H	W	F	Q	Q	K	P
21		I	T	C	R	A	S	Q	S	I	N	T	W	L	A	W	Y	Q	Q	K	P
		<hr/>																			
40		G	T	S	P	K	L	W	I	Y	T	T	S	N	L	A	S	G	V	P	A
41		G	K	A	P	K	L	L	M	Y	K	A	S	S	L	E	S	G	V	P	S
		<hr/>																			
60		R	F	S	G	S	G	S	G	T	S	Y	S	L	T	I	S	R	M	E	A
61		R	F	I	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P
		<hr/>																			
80		E	D	A	A	T	Y	Y	C	H	Q	R	S	T	Y	P	L	T	F	G	S
81		D	D	F	A	T	Y	Y	C	Q	Q	Y	N	S	D	S	K	M	F	G	Q
		<hr/>																			
100		G	T	K	L	E	L	K													
101		G	T	K	V	E	V	K													

FIGURE 3

10 20 30 40 50 60
TCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCTCAGGTACCGCGGGCGTGCACT
M G W S W I F L F L L S G T A G V H

70 80 90 100 110 120
CTEAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTGAAGG
S Q V Q L V Q S G A E V K K P G S S V K

130 140 150 160 170 180
TCTCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACAGGATGCACTGGGTAAGGCAGG
V S C K A S G Y T F T S Y R M H W V R Q

190 200 210 220 230 240
CCCCTGGACAGGGTCTGGAATGGATTGGATATATTAATCCGTCGACTGGGTATACTGAAT
A P G Q G L E W I G Y I N P S T G Y T E

250 260 270 280 290 300
ACAATCAGAAGTTCAAGGACAAGGCAACAATTACTGCAGACGAATCCACCAATACAGCCT
Y N Q K F K D K A T I T A D E S T N T A

310 320 330 340 350 360
ACATGGAAGTCTGAGCAGCCTGAGATCTGAGGACACCGCAGTCTATTACTGTGCAAGAGGGG
Y M E L S S L R S E D T A V Y Y C A R G

370 380 390 400 410 420
GGGGGTCTTTGACTACTGGGGCCAAGGAACCCTGGTCACAGTCTCCTCAGGTGAGTCTCT
G G V F D Y W G Q G T L V T V S S

430
TAAAACCTCTAGA

FIGURE 4

10 20 30 40 50 60
TCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGATCAA
M E T D T L L L W V L L L W V P G S

70 80 90 100 110 120
CCGGAGATATTGAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGATAGGG
T G D I Q M T Q S P S T L S A S V G D R

130 140 150 160 170 180
TCACCATAACCTGCTCTGCCAGCTCAAGTATAAGTTACATGCACTGGTACCAGCAGAAGC
V T I T C S A S S S I S Y M H W Y Q Q K

190 200 210 220 230 240
CAGGCAAAGCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTG
P G K A P K L L I Y T T S N L A S G V P

250 260 270 280 290 300
CTCGCTTCAGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGC
A R F S G S G S G T E F T L T I S S L Q

310 320 330 340 350 360
CAGATGATTTGCGCACTTATTACTGCCATCAAAGGAGTACTTACCCACTCACGTTCCGGTC
P D D F A T Y Y C H Q R S T Y P L T F G

370 380 390 400
AGGGGACCAAGGTGGAGGTCAAACGTAAGTACACTTTTCTAGA
Q G T K V E V K

FIGURE 5

A

HES12 AGCTTCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTG
CACTCTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTG
AAGGTC

HES13 CCCAGTCGACGGATTAATATATCCAATCCATTCCAGACCCTGTCCAGGGGCCTGCCTTAC
CCAGTGCATCCTGTAGCTAGTAAAGGTGTAGCCAGAAGCCTTGCAGGAGACCTTCACGCT
CGAGCCAGG

HES14 TATATTAATCCGTCGACTGGGTATACTGAATACAATCAGAAGTTCAAGGACAAGGCAACA
ATTACTGCAGACGAATCCACCAATACAGCCTACATGGAAGTGAAGCAGCCTGAGATCTGAG
GACA

HES15 ATATCGTCTAGAGGTTTTAAGGACTCACCTGAGGAGACTGTGACCAGGGTTCCTTGGCCC
CAGTAGTCAAAGACCCCCCCCCCTCTGCACAGTAATAGACTGCGGTGTCTCAGATCTC
AGGCTGCT

B

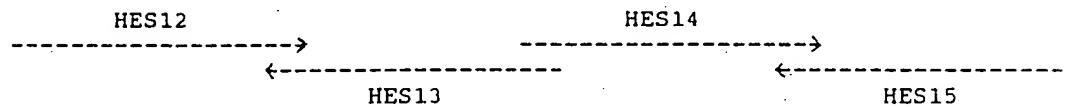


FIGURE 6

A

JFD1 CAAATCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGA
TCAACCGGAGATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGAT

JFD2 ATAAATTAGAAGCTTGGGAGCTTTGCCTGGCTTCTGCTGGTACCAGTGCATGTAACCTTAT
ACTTGAGCTGGCAGAGCAGGTTATGGTGACCCCTATCCCCGACGCTAGCAGAGAG

JFD3 GCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTC
AGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGCCAGATGAT
TTC

JFD4 TATATCTAGAAAAGTGTACTTACGTTTGACCTCCACCTTGGTCCCCTGACCGAACGTGAG
TGGGTAAGTACTCCTTTGATGGCAGTAATAAGTGGCGAAATCATCTGGCTGCAGAGAGCT
GA

B

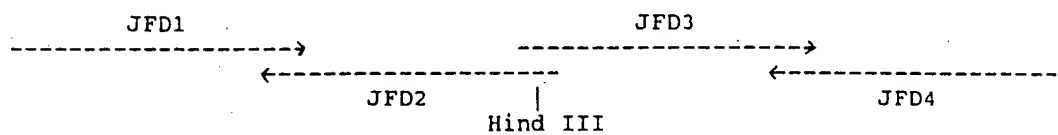


FIGURE 7

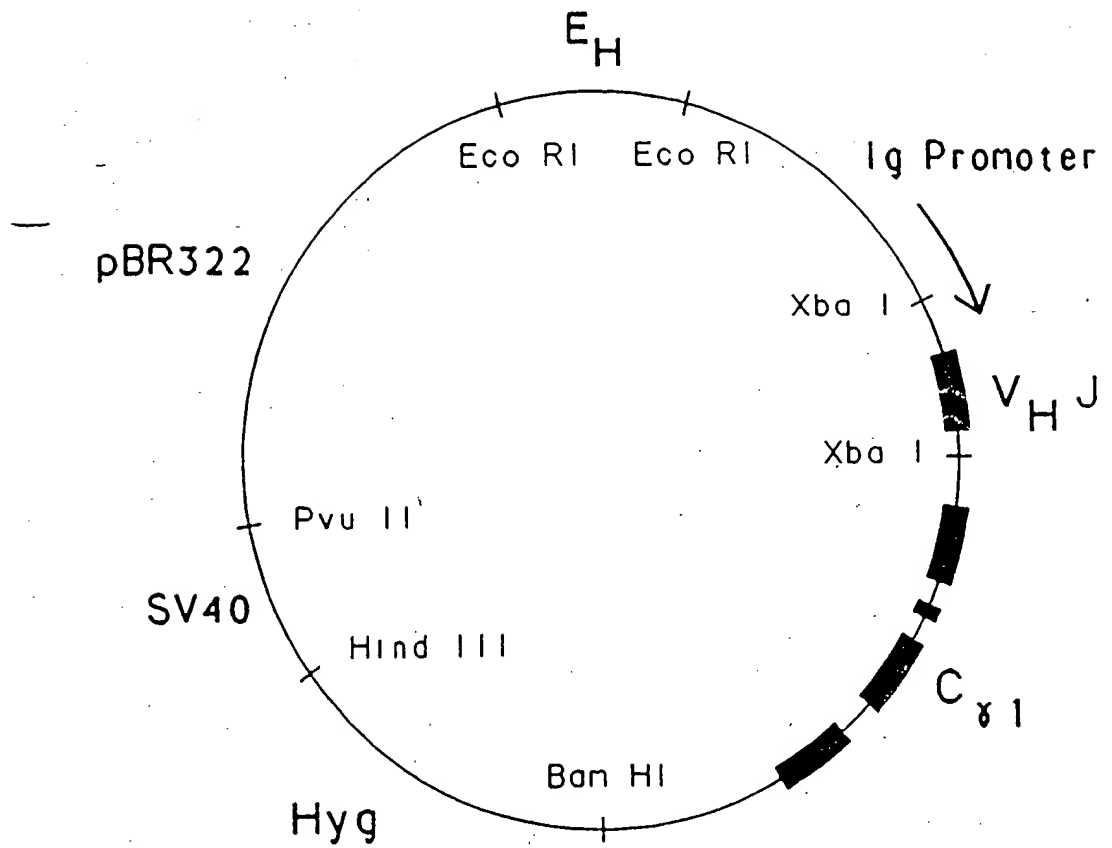


FIGURE 8

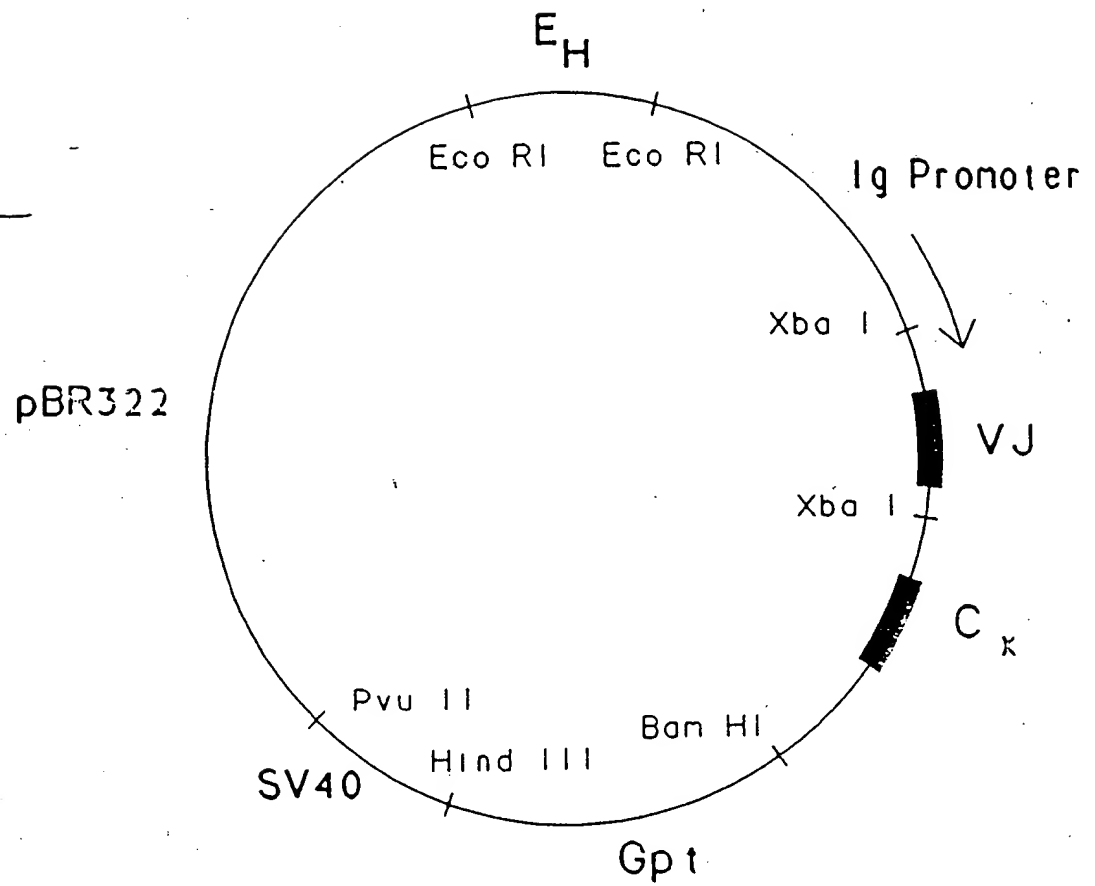


FIGURE 9

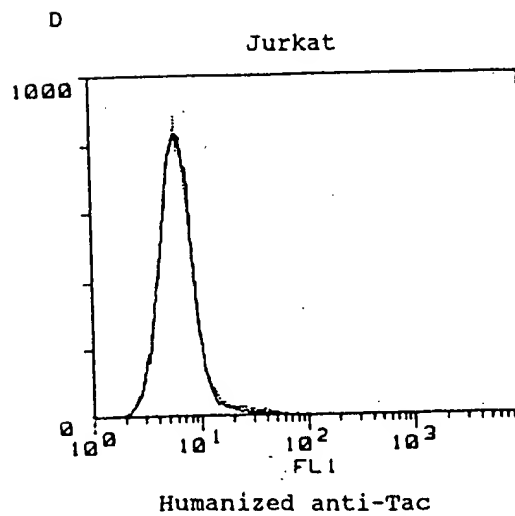
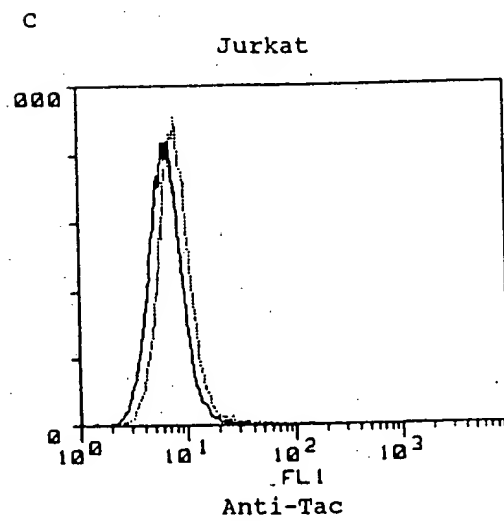
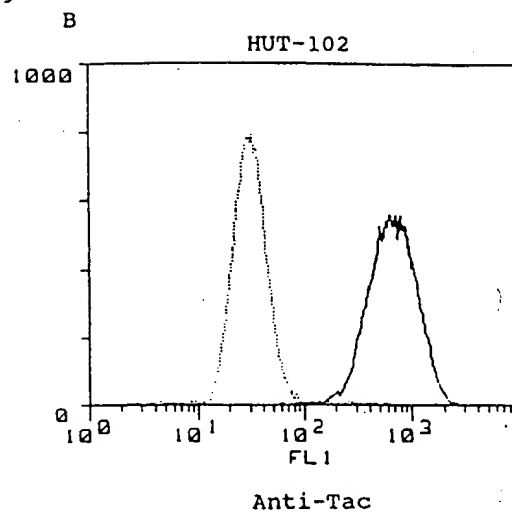
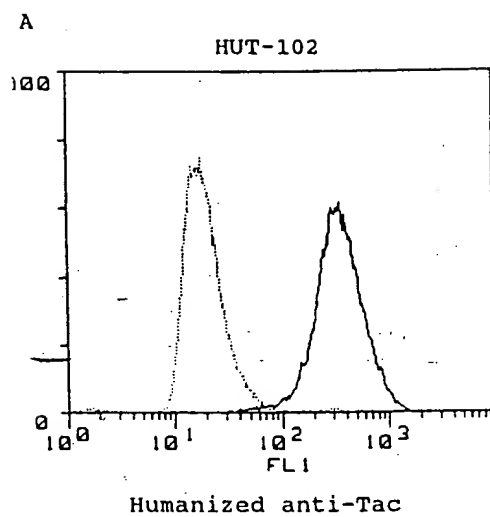
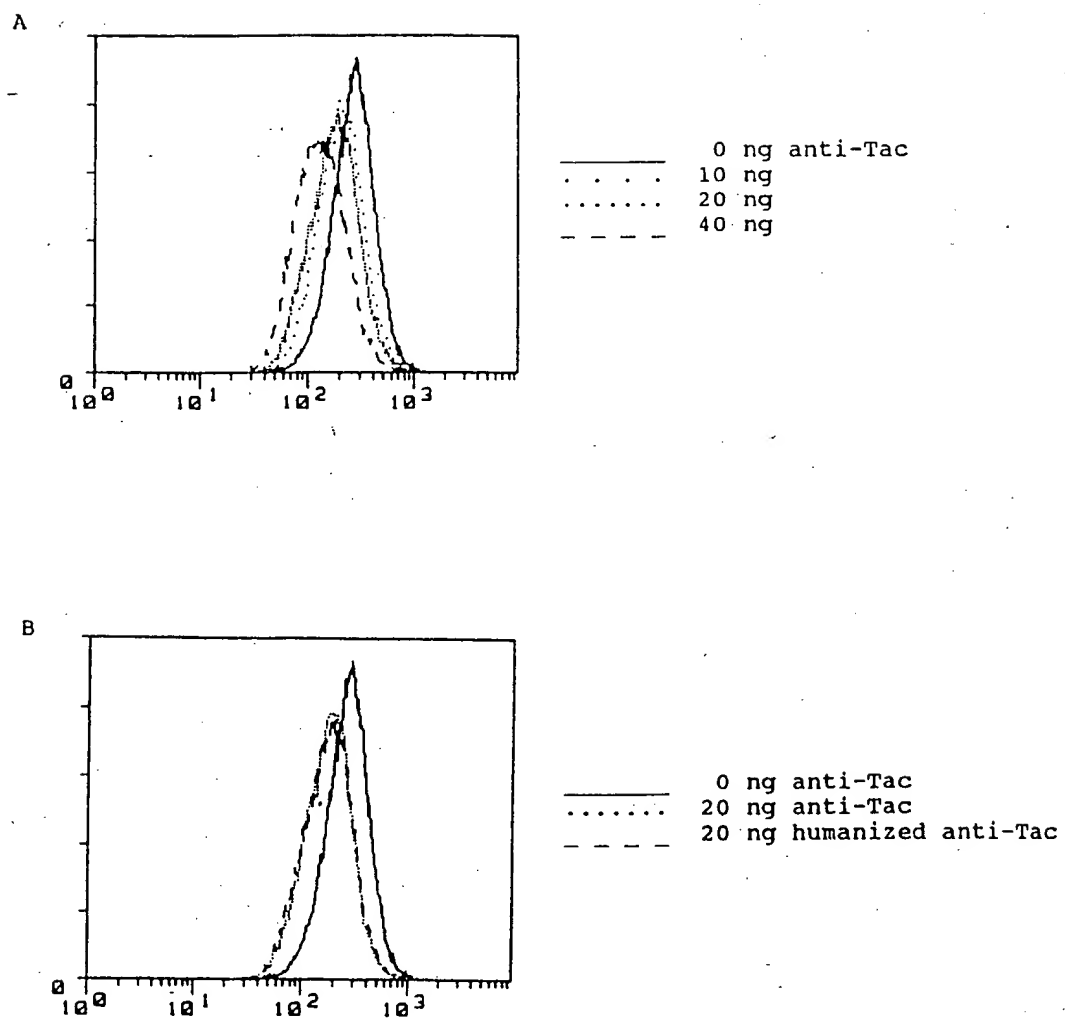


FIGURE 10



310/252

11823-9

DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation-in-part application of
commonly assigned patent application U.S.S.N. 290,975, filed
December 28, 1988, which is incorporated herein by reference.

Field of the Invention

The present invention relates generally to the
combination of recombinant DNA and monoclonal antibody
technologies for developing novel therapeutic agents and,
more particularly, to the production of non-immunogenic
antibodies having strong affinity for a predetermined
antigen.

Background of the Invention

The advent of monoclonal antibody technology in the
mid 1970's heralded a new age of medicine. For the first
time, researchers and clinicians had access to essentially
unlimited quantities of uniform antibodies capable of binding
to a predetermined antigenic site and having various
immunological effector functions. These proteins, known as
"monoclonal antibodies" were thought to hold great promise
in, e.g., the removal of harmful cells in vivo. Indeed, the
clinical value of monoclonal antibodies seemed limitless for
this use alone.

Unfortunately, the development of appropriate
therapeutic products based on these proteins has been
severely hampered by a number of drawbacks inherent in
monoclonal antibody production. For example, most monoclonal
antibodies are mouse derived, and thus do not fix human
complement well. They also lack other important
immunoglobulin functional characteristics when used in
humans.

Perhaps most importantly, non-human monoclonal
antibodies contain substantial stretches of amino acid
sequences that will be immunogenic when injected into a human

patient. Numerous studies have shown that after injection of a foreign antibody, the immune response mounted by a patient can be quite strong, essentially eliminating the antibody's therapeutic utility after an initial treatment. Moreover, as increasing numbers of different mouse or other antigenic (to humans) monoclonal antibodies can be expected to developed to treat various diseases, after the first or second treatments with any non-human antibodies, subsequent treatments, even for unrelated therapies, can be ineffective or even dangerous in themselves.

While the production of so called "chimeric antibodies" (e.g., mouse variable regions joined to human constant regions) has proven somewhat successful, a significant immunogenicity problem remains. Moreover, efforts to immortalize human B-cells or generate human hybridomas capable of producing human immunoglobulins against a desired antigen have been generally unsuccessful, particularly with many important human antigens. Most recently, recombinant DNA technology has been utilized to produce immunoglobulins which have human framework regions combined with complementarity determining regions (CDR's) from a donor mouse or rat immunoglobulin (see, e.g., EPO Publication No. 0239400, which is incorporated herein by reference). These new proteins are called "humanized immunoglobulins" and the process by which the donor immunoglobulin is converted into a human-like immunoglobulin by combining its CDR's with a human framework is called "humanization". Humanized antibodies are important because they bind to the same antigen as the original antibodies, but are less immunogenic when injected into humans.

However, a major problem with present humanization procedures has been a loss of affinity for the antigen, usually by at least 2 to 3-fold (Jones et al., Nature, 321:522-525 (1986)) and in some instances as much as 10-fold or more, especially when the antigen is a protein (Verhoeyen et al., Science, 239:1534-1536 (1988)). Loss of any affinity is, of course, highly undesirable. At the least, it means that more of the humanized antibody will have to be injected

into the patient, at higher cost and greater risk of adverse effects. Even more critically, an antibody with reduced affinity may have poorer biological functions, such as complement lysis, antibody-dependent cellular cytotoxicity, or virus neutralization. For example, the loss of affinity in the partially humanized antibody HuVHCAMP may have caused it to lose all ability to mediate complement lysis (see, Riechmann et al., Nature, 332:323-327 (1988); Table 1).

Thus, there is a need for improved means for producing humanized antibodies specifically reactive with strong affinity to a predetermined antigen. These humanized immunoglobulins should remain substantially non-immunogenic in humans, yet be easily and economically produced in a manner suitable for therapeutic formulation and other uses. The present invention fulfills these and other needs.

Summary of the Invention

The present invention provides novel methods for designing humanized immunoglobulin chains having one or more complementarity determining regions (CDR's) from a donor immunoglobulin and a framework region from a human immunoglobulin, the preferred methods comprising first comparing the framework or variable region amino acid sequence of the donor immunoglobulin to corresponding sequences in a collection of human immunoglobulin chains, and selecting as the human immunoglobulin one of the more homologous sequences from the collection. The human immunoglobulin, or acceptor immunoglobulin, sequence is typically selected from a collection of at least 10 to 20 immunoglobulin chain sequences, and usually will have the highest homology to the donor immunoglobulin sequence of any sequence in the collection. The human immunoglobulin framework sequence will typically have about 65 to 70% homology or more to the donor immunoglobulin framework sequences. The donor immunoglobulin may be either a heavy chain or light chain (or both), and the human collection will contain the same kind of chain. A humanized light and heavy chain can be used to form a complete humanized immunoglobulin

or antibody, having two light/heavy chain pairs, with or without partial or full-length human constant regions and other proteins.

In another embodiment of the present invention, either in conjunction with the above comparison step or separately, additional amino acids in an acceptor immunoglobulin chain may be replaced with amino acids from the CDR-donor immunoglobulin chain. More specifically, further optional substitutions of a human framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from a donor immunoglobulin will be made at positions in the immunoglobulins where:

(a) the amino acid in the human framework region of an acceptor immunoglobulin is rare for that position and the corresponding amino acid in the donor immunoglobulin is common for that position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to be within about 3Å of the CDR's in a three-dimensional immunoglobulin model and capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.

The humanized immunoglobulin chain will typically comprise at least about 3 amino acids from the donor immunoglobulin in addition to the CDR's, usually at least one of which is immediately adjacent to a CDR in the donor immunoglobulin. The heavy and light chains may each be designed by using any one or all three of the position criteria.

When combined into an intact antibody, the humanized light and heavy chains of the present invention will be substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen (such as a protein or other compound containing an epitope). These affinity levels can vary from about 10^8 M^{-1} or higher, and may be within about 4 fold of the donor immunoglobulin's original affinity to the antigen.

Once designed, the immunoglobulins, including binding fragments and other immunoglobulin forms, of the present invention may be produced readily by a variety of recombinant DNA or other techniques. Preferably, polynucleotides encoding the desired amino acid sequences are produced synthetically or by joining appropriate nucleic acid sequences for expression in a suitable host (e.g., cell culture). The humanized immunoglobulins will be particularly useful in treating human disorders susceptible to monoclonal antibody therapy, but find a variety of other uses as well.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Comparison of sequences of anti-Tac heavy chain (upper lines) and Eu heavy chain (lower lines). The 1-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDR's are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an *.

Figure 2. Comparison of sequences of anti-Tac light chain (upper lines) and Eu light chain (lower lines). The single-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDR's are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an *.

Figure 3. Nucleotide sequence of the gene for the humanized anti-Tac heavy chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature heavy chain sequence begins with amino acid #20 Q.

Figure 4. Nucleotide sequence of the gene for the humanized anti-Tac light chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature light chain sequence begins with amino acid #21 D.

Figure 5. A. Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac heavy chain gene, printed 5' to 3'. B. Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide.

Figure 6. (A) Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac light chain gene, printed 5' to 3'. (B) Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide. The position of a Hind III site in the overlap of JFD2 and JFD3 is shown.

Figure 7. Schematic diagram of the plasmid pHuGTAC1 used to express the humanized anti-Tac heavy chain. Relevant restriction sites are shown, and coding regions of the heavy chain are displayed as boxes. The direction of transcription from the immunoglobulin (Ig) promoter is shown by an arrow. E_H = heavy chain enhancer, Hyg = hygromycin resistance gene.

Figure 8. Schematic diagram of the plasmid pHuLTAC used to express the humanized anti-Tac light chain. Relevant restriction sites are shown, and coding regions of the light chain are displayed as boxes. The direction of transcription from the Ig promoter is shown by an arrow.

Figure 9. Fluorocytometry of HUT-102 and Jurkat cells stained with anti-Tac antibody or humanized anti-Tac antibody followed respectively by fluorescein-conjugated goat anti-mouse Ig antibody or goat anti-human Ig antibody, as labeled. In each panel, the dotted curve shows the results when the first antibody was omitted, and the solid curve the results when first and second (conjugated) antibodies were included as described.

Figure 10. (A) Fluorocytometry of HUT-102 cells stained with 0-40 ng of anti-Tac as indicated, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin. (B) Fluorocytometry of HUT-102 cells stained with the indicated antibody, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, novel means of designing humanized immunoglobulins capable of specifically binding to a predetermined antigen with strong affinity are provided. These improved methods produce immunoglobulins that are substantially non-immunogenic in humans but have binding affinities of at least about 10^8 M^{-1} , preferably 10^9 M^{-1} to 10^{10} M^{-1} , or stronger. The humanized immunoglobulins will have a human framework and have one or more complementary determining regions (CDR's), plus a limited number of other amino acids, from a donor immunoglobulin specifically reactive with an antigen. The immunoglobulins can be produced economically in large quantities and find use, for example, in the treatment of various human disorders by a variety of techniques.

In order that the invention may be more completely understood, several definitions are set forth. As used herein, the term "immunoglobulin" refers to a protein having one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 Kd, about 214 amino acids) are encoded by a variable region gene at the NH₂-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH - terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd, about 446 amino acids), are similarly encoded by a variable region gene (encoding about 116 amino acids) and one of the other

aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

One form of immunoglobulin constitutes the basic structural unit of an antibody. This form is a tetramer and consists of two identical pairs of immunoglobulin chains, each pair having one light and one heavy chain. In each pair, the light and heavy chain variable regions are together responsible for binding to an antigen, and the constant regions are responsible for the antibody effector functions. In addition to antibodies, immunoglobulins may exist in a variety of other forms (including less than full-length that retain the desired activities), including, for example, Fv, Fab, and F(ab')₂, as well as single chain antibodies (e.g., Huston et al., Proc. Nat. Acad. Sci. U.S.A., 85:5879-5883 (1988) and Bird et al., Science, 242:423-426 (1988), which are incorporated herein by reference). (See, generally, Hood et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, Nature, 323:15-16 (1986), which are incorporated herein by reference).

An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, also called CDR's. The extent of the framework region and CDR's have been precisely defined (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services, (1983); which is incorporated herein by reference). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDR's. The CDR's are primarily responsible for binding to an epitope of an antigen.

Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant region genes belonging to different species. For example, the variable segments of the genes from a mouse monoclonal antibody may be joined to human constant segments, such as

gamma 1 and gamma 3. A typical therapeutic chimeric antibody is thus a hybrid protein composed of the variable or antigen-binding domain from a mouse antibody and the constant or effector domain from a human antibody (e.g., A.T.C.C. Accession No. CRL 9688 secretes an anti-Tac chimeric antibody), although other mammalian species may be used.

As used herein, the term "humanized" immunoglobulin refers to an immunoglobulin comprising a substantially human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the "donor" and a human immunoglobulin providing the framework is called the "acceptor". Constant regions need not be present, but if they are, they must be substantially homologous to human immunoglobulin constant regions, i.e., at least about 85-90%, preferably about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's, are substantially homologous to corresponding parts of natural human immunoglobulin sequences. A "humanized antibody" is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. For example, a humanized antibody would not encompass a typical chimeric antibody as defined above, e.g., because the entire variable region of a chimeric antibody is non-human. One says that the donor antibody has been "humanized", by the process of "humanization", because the resultant humanized antibody is expected to bind to the same antigen as the donor antibody that provides the CDR's.

Humanized immunoglobulins, including humanized antibodies, have been constructed by means of genetic engineering. Most humanized immunoglobulins that have been previously described (Jones et al., op. cit.; Verhoeyen et al., op. cit.; Riechmann et al., op. cit.) have comprised a framework that is identical to the framework of a particular human immunoglobulin chain, the acceptor, and three CDR's from a non-human donor immunoglobulin chain. In one case (Riechmann et al., op. cit.), two additional amino acids in the framework were changed to be the same as amino acids in

other human framework regions. The present invention includes criteria by which a limited number of amino acids in the framework of a humanized immunoglobulin chain are chosen to be the same as the amino acids at those positions in the donor rather than in the acceptor, in order to increase the affinity of an antibody comprising the humanized immunoglobulin chain.

The present invention is based in part on the model that two contributing causes of the loss of affinity in prior means of producing humanized antibodies (using as examples mouse antibodies as the source of CDR's) are:

(1) When the mouse CDR's are combined with the human framework, the amino acids in the framework close to the CDR's become human instead of mouse. Without intending to be bound by theory, we believe that these changed amino acids may slightly distort the CDR's, because they create different electrostatic or hydrophobic forces than in the donor mouse antibody, and the distorted CDR's may not make as effective contacts with the antigen as the CDR's did in the donor antibody;

(2) Also, amino acids in the original mouse antibody that are close to, but not part of, the CDR's (*i.e.*, still part of the framework), may make contacts with the antigen that contribute to affinity. These amino acids are lost when the antibody is humanized, because all framework amino acids are made human.

To avoid these problems, and to produce humanized antibodies that have a very strong affinity for a desired antigen, the present invention uses the following four criteria for designing humanized immunoglobulins. These criteria may be used singly, or when necessary in combination, to achieve the desired affinity or other characteristics.

Criterion I: As acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. For example,

comparison of the sequence of a mouse heavy (or light) chain variable region against human heavy (or light) variable regions in a data bank (for example, the National Biomedical Research Foundation Protein Identification Resource) shows that the extent of homology to different human regions varies greatly, typically from about 40% to about 60-70%. By choosing as the acceptor immunoglobulin one of the human heavy (respectively light) chain variable regions that is most homologous to the heavy (respectively light) chain variable region of the donor immunoglobulin, fewer amino acids will be changed in going from the donor immunoglobulin to the humanized immunoglobulin. Hence, and again without intending to be bound by theory, it is believed that there is a smaller chance of changing an amino acid near the CDR's that distorts their conformation. Moreover, the precise overall shape of a humanized antibody comprising the humanized immunoglobulin chain may more closely resemble the shape of the donor antibody, also reducing the chance of distorting the CDR's.

Typically, one of the 3-5 most homologous heavy chain variable region sequences in a representative collection of at least about 10 to 20 distinct human heavy chains will be chosen as acceptor to provide the heavy chain framework, and similarly for the light chain. Preferably, one of the 1-3 most homologous variable regions will be used. The selected acceptor immunoglobulin chain will most preferably have at least about 65% homology in the framework region to the donor immunoglobulin.

Regardless of how the acceptor immunoglobulin is chosen, higher affinity may be achieved by selecting a small number of amino acids in the framework of the humanized immunoglobulin chain to be the same as the amino acids at those positions in the donor rather than in the acceptor. The following criteria define what amino acids may be so selected. Preferably, at most or all amino acid positions satisfying one of these criteria, the donor amino acid will in fact be selected.

Criterion II: If an amino acid in the framework of the human acceptor immunoglobulin is unusual (i.e., "rare", which as used herein indicates an amino acid occurring at that position in no more than about 10% of human heavy

(respectively light) chain V region sequences in a representative data bank), and if the donor amino acid at that position is typical for human sequences (i.e., "common", which as used herein indicates an amino acid occurring in at least about 25% of sequences in a representative data bank), then the donor amino acid rather than the acceptor may be selected. This criterion helps ensure that an atypical amino acid in the human framework does not disrupt the antibody structure. Moreover, by replacing an unusual amino acid with an amino acid from the donor antibody that happens to be typical for human antibodies, the humanized antibody may be made less immunogenic.

Criterion III: In the positions immediately adjacent to the 3 CDR's in the humanized immunoglobulin chain, the donor amino acid rather than acceptor amino acid may be selected. These amino acids are particularly likely to interact with the amino acids in the CDR's and, if chosen from the acceptor, distort the donor CDR's and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit et al., Science, 233, 747-753 (1986), which is incorporated herein by reference) and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

Criterion IV: A 3-dimensional model, typically of the original donor antibody, shows that certain amino acids outside of the CDR's are close to the CDR's and have a good probability of interacting with amino acids in the CDR's by hydrogen bonding, Van der Waals forces, hydrophobic interactions, etc. At those amino acid positions, the donor amino acid rather than the acceptor immunoglobulin amino acid may be selected. Amino acids according to this criterion will

generally have a side chain atom within about 3 angstrom units of some site in the CDR's and must contain atoms that could interact with the CDR atoms according to established chemical forces, such as those listed above. Computer programs to create models of proteins such as antibodies are generally available and well known to those skilled in the art (see, Loew *et al.*, Int. J. Quant. Chem., Quant. Biol. Symp., 15:55-66 (1988); Bruccoleri *et al.*, Nature, 335, 564-568 (1988); Chothia *et al.*, Science, 233:755-758 (1986), all of which are incorporated herein by reference). These do not form part of the invention. Indeed, because all antibodies have similar structures, the known antibody structures, which are available from the Brookhaven Protein Data Bank, can be used if necessary as rough models of other antibodies. Commercially available computer programs can be used to display these models on a computer monitor, to calculate the distance between atoms, and to estimate the likelihood of different amino acids interacting (see, Ferrin *et al.*, J. Mol. Graphics, 6:13-27 (1988)).

Humanized antibodies generally have at least three potential advantages over mouse or in some cases chimeric antibodies for use in human therapy:

1) Because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).

2) The human immune system should not recognize the framework or constant region of the humanized antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign mouse antibody or a partially foreign chimeric antibody.

3) Injected mouse antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of normal antibodies (D. Shaw *et al.*, J. Immunol., 138:4534-4538 (1987)). Injected humanized antibodies will presumably have a half-life more similar to

naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

In one aspect, the present invention is directed to designing humanized immunoglobulins that are produced by expressing recombinant DNA segments encoding the heavy and light chain CDR's from a donor immunoglobulin capable of binding to a desired antigen, such as the human IL-2 receptor, to DNA segments encoding acceptor human framework regions. Exemplary DNA sequences designed in accordance with the present invention and, which on expression code for the polypeptide chains comprising heavy and light chain CDR's with substantially human framework regions, are shown in Figures 3 and 4, respectively. Due to codon degeneracy and non-critical amino acid substitutions, other DNA sequences can be readily substituted for those sequences, as detailed below. In general, the criteria of the present invention find applicability to designing substantially any humanized immunoglobulin.

The DNA segments will typically further include an expression control DNA sequence operably linked to the humanized immunoglobulin coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the humanized light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow (see, S. Beychok, Cells of Immunoglobulin Synthesis, Academic Press, N.Y., (1979), which is incorporated herein by reference.

Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B-cells (see, Kabat

op. cit. and WP87/02671). The CDR's for producing the immunoglobulins of the present invention will be similarly derived from monoclonal antibodies capable of binding to the predetermined antigen, such as the human IL-2 receptor, and produced by well known methods in any convenient mammalian source including, mice, rats, rabbits, or other vertebrate capable of producing antibodies. Suitable source cells for the constant region and framework DNA sequences, and host cells for immunoglobulin expression and secretion, can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference).

In addition to the humanized immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins to the native sequences can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary specifically from the sequences in Figures 3 and 4 at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Gillman and Smith, Gene, 8:81-97 (1979) and S. Roberts et al., Nature, 328:731-734 (1987), both of which are incorporated herein by reference).

Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). Also because like many genes, the immunoglobulin-related genes contain separate functional regions, each having one or more distinct biological activities, the genes may be fused to functional regions from other genes (e.g., enzymes, see,

commonly assigned U.S.S.N. 132,387, filed Dec. 15, 1987, which is incorporated herein by reference) to produce fusion proteins (e.g., immunotoxins) having novel properties. The nucleic acid sequences of the present invention capable of ultimately expressing the desired humanized antibodies can be
5 formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), as well as by a variety of different techniques. Joining appropriate genomic sequences is presently the most common method of production,
10 but cDNA sequences may also be utilized (see, European Patent Publication No. 0239400 and L. Reichmann et al., Nature, 312:323-327 (1988), both of which are incorporated herein by reference).

As stated previously, the DNA sequences will be
15 expressed in hosts after the sequences have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly,
20 expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences (see, e.g., U.S. Patent 4,704,362, which is incorporated herein by reference).

E. coli is one prokaryotic host useful particularly
25 for cloning the DNA sequences of the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression
30 vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (*trp*) promoter system, a beta-lactamase
35 promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site

sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may also be used for expression. *Saccharomyces* is a preferred host, with suitable vectors having expression control sequences, such as promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (see, Winnacker, "From Genes to Clones," VCH Publishers, N.Y., N.Y. (1987), which is incorporated herein by reference). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed in the art, and include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, etc, but preferably transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen et al., *Immunol. Rev.*, 89:49-68 (1986), which is incorporated herein by reference), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, and the like.

The vectors containing the DNA segments of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts. (See, generally, Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, (1982), which is incorporated herein by reference.)

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, R. Scopes, "Protein Purification", Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures, immunofluorescent stainings, and the like. (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The antibodies of the present invention will typically find use individually in treating substantially any disease susceptible to monoclonal antibody-based therapy. In particular, the immunoglobulins can be used for passive immunization or the removal of unwanted cells or antigens, such as by complement mediated lysis, all without substantial immune reactions (e.g., anaphylactic shock) associated with many prior antibodies. For example, where the cell linked to a disease has been identified as IL-2 receptor bearing, then humanized antibodies that bind to the human IL-2 receptor are suitable (see, U.S.S.N. 085,707, entitled "Treating Human Malignancies and Disorders," which is incorporated herein by reference). For such a humanized immunoglobulin, typical disease states suitable for treatment include graft versus host disease and transplant rejection in patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc. Other diseases include autoimmune diseases, such as Type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis.

The method of producing humanized antibodies of the present invention can be used to humanize a variety of donor

antibodies, especially monoclonal antibodies reactive with markers on cells responsible for a disease. For example, suitable antibodies bind to antigens on T-cells, such as those grouped into the so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Bernard et al., Eds., Springer-Verlag, N.Y. (1984), which is incorporated herein by reference.

The antibodies of the present invention can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

A preferred pharmaceutical composition of the present invention comprises the use of the subject antibodies in immunotoxins. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle," provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al., Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), which is incorporated herein by reference.

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides,

such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). (See, generally, commonly assigned U.S.S.N. 290,968 (Townsend and Townsend Docket No. 11823-7-2) filed in U.S.P.T.O. on December 28, 1988, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985), all of which are incorporated herein by reference.)

The delivery component of the immunotoxin will include the humanized immunoglobulins of the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

For diagnostic purposes, the antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the humanized antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme co-factors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

The following examples are offered by way of illustration, not by limitation.

EXPERIMENTAL

Design of genes for humanized light and heavy chains

The sequence of the human antibody Eu (Sequences of Proteins of Immunological Interest, E. Kabat et al., U.S. Dept. of Health and Human Services, 1983) was used to provide the framework of the humanized antibody, because the amino acid sequence of the heavy chain variable region of anti-Tac is more homologous to the heavy chain of this antibody than to any other complete heavy chain variable region sequence in the National Biomedical Foundation Protein Identification Resource.

To select the sequence of the humanized heavy chain, the anti-Tac heavy chain sequence (see, commonly assigned U.S.S.N.'s 186,862 and 223,037, which are incorporated herein by reference) was aligned with the sequence of the Eu heavy chain (Figure 1). At each position, the Eu amino acid was selected for the humanized sequence, unless that position fell in any one of the following categories, in which case the anti-Tac amino acid was selected:

(1) The position fell within a complementarity determining region (CDR), as defined by Kabat, et al., op. cit. (amino acids 31-35, 50-66, 99-106);

(2) The Eu amino acid was rare for human heavy chains at that position, whereas the anti-Tac amino acid was common for human heavy chains at that position (amino acids 27, 93, 95, 98, 107-109, 111);

(3) The position was immediately adjacent to a CDR in the amino acid sequence of the anti-Tac heavy chain (amino acids 30 and 67); or

(4) 3-dimensional modeling of the anti-Tac antibody suggested that the amino acid was physically close to the antigen binding region (amino acids 48 and 68).

Amino acid #27 is listed in category (4) because the acceptor Eu amino acid Gly is rare, and the donor anti-Tac amino acid Tyr is chemically similar to the amino acid Phe, which is common, but the substitution was actually made because #27

also fell in category (4). Although some amino acids fell in more than one of these categories, they are only listed in one. Categories (2) - (4) correspond to criteria (2) - (4) described above.

To select the sequence of the humanized light chain, the anti-Tac light chain sequence was aligned with the sequence of the Eu light chain (Figure 2). The Eu amino acid was selected at each position, unless the position again fell into one of the categories (1) - (4) (with light chain replacing heavy chain in the category definitions):

- (1) CDR's (amino acids 24-34, 50-56, 89-97);
- (2) Anti-Tac amino acid more typical than Eu (amino acids 48 and 63);
- (3) Adjacent to CDR's (no amino acids; Eu and anti-Tac were already the same at all these positions); or
- (4) Possible 3-dimensional proximity to binding region (amino acid 60).

The actual nucleotide sequence of the heavy (Figure 3) and light chain (Figure 4) genes were selected as follows:

- (1) The nucleotide sequences code for the amino acid sequences chosen as described above;
- (2) 5' of these coding sequences, the nucleotide sequences code for a leader (signal) sequence, namely the leader of the light chain of the antibody MOPC 63 and the leader of the heavy chain of the antibody PCH 108A (Kabat et al., op. cit.). These leader sequences were chosen as typical of antibodies;
- (3) 3' of the coding sequences, the nucleotide sequences are the sequences that follow the mouse light chain J5 segment and the mouse heavy chain J2 segment, which are part of the anti-Tac sequences. These sequences are included because they contain splice donor signals; and
- (4) At each end of the sequence is an Xba I site to allow cutting at the Xba I sites and cloning into the Xba I site of a vector.

Construction of humanized light and heavy chain genes

To synthesize the heavy chain, four oligonucleotides HES12, HES13, HES14, HES15 (Figure 5A) were synthesized using an Applied Biosystems 380B DNA synthesizer. Two of the oligonucleotides are part of each strand of the heavy chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 5B). Together, the oligonucleotides cover the entire humanized heavy chain variable region (Figure 3) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

Each oligonucleotide was phosphorylated using ATP and T4 polynucleotide kinase by standard procedures (see, Maniatis, op. cit.). To anneal the phosphorylated oligonucleotides, they were suspended together in 40 ul of TA (33 mM Tris acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate) at a concentration of about 3.75 uM each, heated to 95 deg for 4 min. and cooled slowly to 4 deg. To synthesize the complete gene from the oligonucleotides by synthesizing the opposite strand of each oligonucleotide (Figure 5B), the following components were added in a final volume of 100ul:

10 ul	annealed oligonucleotides
0.16 mM each	deoxyribonucleotide
0.5 mM	ATP
0.5 mM	DTT
100 ug/ml	BSA
3.5 ug/ml	T4 g43 protein (DNA polymerase)
25 ug/ml	T4 g44/62 protein (polymerase accessory protein)
25 ug/ml	45 protein (polymerase accessory protein)

The mixture was incubated at 37 deg for 30 min. Then 10 u of T4 DNA ligase was added and incubation at 37 deg resumed for 30 min. The polymerase and ligase were inactivated by incubation of the reaction at 70 deg for

15 min. To digest the gene with Xba I, to the reaction was added 50 ul of 2x TA containing BSA at 200 ug/ml and DTT at 1 mM, 43 ul of water, and 50 u of Xba I in 5 ul. The reaction was incubated for 3 hr at 37 deg, and run on a gel. The 431 bp Xba I fragment was purified from a gel and cloned into the Xba I site of the plasmid pUC19 by standard methods. Four plasmid isolates were purified and sequenced using the dideoxy method. One of these had the correct sequence (Figure 3).

To synthesize the light chain, four oligonucleotides JFD1, JFD2, JFD3, JFD4 (Figure 6A) were synthesized. Two of the oligonucleotides are part of each strand of the light chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 6B). Together, the oligonucleotides cover the entire humanized light chain variable region (Figure 4) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

The light chain gene was synthesized from these oligonucleotides in two parts. 0.5 ug each of JFD1 and JFD2 were combined in 20 ul sequenase buffer (40 mM Tris-HCl, pH 7.5, 20 mM magnesium chloride, 50 mM sodium chloride), heated at 70 deg for 3 min and allowed to cool slowly to 23 deg in order for the oligonucleotides to anneal. JFD3 and JFD4 were treated in the same way. Each reaction was made 10 mM in DTT and 0.5 mM in each deoxyribonucleotide and 6.5 u of sequenase (US Biochemicals) was added, in a final volume of 24 ul, and incubated for 1 hr at 37 deg to synthesize the opposite strands of the oligonucleotides. Xba I and Hind III were added to each reaction to digest the DNA (there is a Hind III site in the region where JFD2 and JFD3 overlap and therefore in each of the synthesized DNAs; Figure 6B). The reactions were run on polyacrylamide gels, and the Xba I - Hind III fragments were purified and cloned into pUC18 by standard methods. Several plasmid isolates for each fragment were sequenced by the dideoxy method, and correct ones chosen.

Construction of plasmids to express humanized light and heavy chains

The heavy chain Xba I fragment was isolated from the pUC19 plasmid in which it had been inserted and then inserted into the Xba I site of the vector pVγ1 (see, commonly assigned U.S.S.N. 223,037) in the correct orientation by standard methods, to produce the plasmid pHuGTAC1 (Figure 7). This plasmid will express high levels of a complete heavy chain when transfected into an appropriate host cell.

The two light chain Xba I - Hind III fragments were isolated from the pUC18 plasmids in which they had been inserted. The vector plasmid pVκ1 (see, commonly assigned U.S.S.N. 223,037) was cut with Xba I, dephosphorylated and ligated with the two fragments by standard methods. The desired reaction product has the circular form: vector - Xba I - fragment 1 - Hind III - fragment 2 - Xba I - vector. Several plasmid isolates were analyzed by restriction mapping and sequencing, and one with this form chosen. This plasmid, pHuLTAC (Figure 8), therefore contains the complete humanized light chain (Figure 4) and will express high levels of the light chain when transfected into an appropriate host cell.

Synthesis and affinity of humanized antibody

The plasmids pHuGTAC1 and pHuLTAC were transfected into mouse Sp2/0 cells, and cells that integrated the plasmids were selected on the basis of resistance to mycophenolic acid and/or hygromycin B conferred by the gpt and hyg genes on the plasmids (Figures 7,8) by standard methods. To verify that these cells secreted antibody that binds to the IL-2 receptor, supernatant from the cells was incubated with HUT-102 cells that are known to express the IL-2 receptor. After washing, the cells were incubated with fluorescein-conjugated goat anti-human antibody, washed, and analyzed for fluorescence on a FACSCAN cytofluorometer. The results (Figure 9A), clearly show that the humanized antibody binds to these cells, but not to Jurkat T-cells that do not express the IL-2 receptor (Figure 9D). As controls, the

original mouse anti-Tac antibody was also used to stain these cells (Figure 9B,C), giving similar results.

For further experiments, cells producing the humanized antibody were injected into mice, and the resultant ascites collected. Humanized antibody was purified to
5 substantial homogeneity from the ascites by passage through an affinity column of goat anti-human immunoglobulin antibody, prepared on an Affigel-10 support (Bio-Rad Laboratories, Inc., Richmond, CA) according to standard techniques. To determine the affinity of the humanized
10 antibody relative to the original anti-Tac antibody, a competitive binding experiment was performed. About 5×10^5 HUT-102 cells were incubated with known quantities (10 - 40 ng) of the anti-Tac antibody and the humanized anti-Tac antibody for 10 min at 4 deg. Then 100 ng of biotinylated
15 anti-Tac was added to the cells and incubated for 30 min at 4 deg. This quantity of anti-Tac had previously been determined to be sufficient to saturate the binding sites on the cells, but not to be in large excess. Then the cells were washed twice with 2 ml of phosphate buffered saline
20 (PBS) containing 0.1% sodium azide. The cells were then incubated for 30 min at 4 deg with 250 ng of phycoerythrin-conjugated avidin, which bound to the biotinylated anti-Tac already bound to the cells. The cells were washed again as above, fixed in PBS containing 1%
25 paraformaldehyde, and analyzed for fluorescence on a FACSCAN cytofluorometer.

Use of increasing amounts (10 - 40 ng) of the anti-Tac antibody as competitor in the first step decreased
30 the amount of biotinylated anti-Tac that could bind to the cells in the second step, and therefore the amount of phycoerythrin-conjugated avidin that bound in the last step, thus decreasing fluorescence (Figure 10A). Equivalent amounts (20 ng) of anti-Tac, and humanized anti-Tac used as competitor decreased the fluorescence to approximately the
35 same degree (Figure 10B). This shows that these antibodies have approximately the same affinity, because if one had greater affinity, it would have more effectively competed

with the biotinylated anti-Tac, thus decreasing fluorescence more.

Biological properties of the humanized antibody

For optimal use in treatment of human disease, the humanized antibody should be able to destroy T-cells in the body that express the IL-2 receptor. One mechanism by which antibodies may destroy target cells is antibody-dependent cell-mediated cytotoxicity, abbreviated ADCC (Fundamental Immunology, Paul, W., Ed., Raven Press, New York (1984), at pg. 681), in which the antibody forms a bridge between the target cell and an effector cell such as a macrophage that can lyse the target. To determine whether the humanized antibody and the original mouse anti-Tac antibody can mediate ADCC, a chromium release assay was performed by standard methods. Specifically, human leukemia HUT-102 cells, which express the IL-2 receptor, were incubated with ^{51}Cr to allow them to absorb this radionuclide. The HUT-102 cells were then incubated with an excess of either anti-Tac or humanized anti-Tac antibody. The HUT-102 cells were next incubated for 4 hrs with either a 30:1 or 100:1 ratio of effector cells, which were normal purified human peripheral blood mononuclear cells that had been activated by incubation for about 20 hrs with human recombinant IL-2. Release of ^{51}Cr , which indicated lysis of the target HUT-102 cells, was measured and the background subtracted (Table 1). The results show that at either ratio of effector cells, anti-Tac did not lyse a significant number of the target cells (less than 5%), while the humanized antibody did (more than 20%). Hence, the humanized antibody is likely to be more efficacious than the original mouse antibody in treating T-cell leukemia or other T-cell mediated diseases.

TABLE 1

Percent ^{51}Cr release after ADCC

	<u>Effector: Target ratio</u>	
	30:1	100:1

<u>Antibody</u>		
Anti-Tac	4%	< 1%
Humanized anti-Tac	24%	23%

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

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WE CLAIM:

Sub A1
1. A method of designing a humanized immunoglobulin (Ig) chain having one or more complementarity determining regions (CDR's) from a donor Ig and a framework region from a human Ig, said method comprising: comparing the framework or variable region amino acid sequence of the donor Ig with corresponding sequences in a collection of human Ig chains; and selecting to provide the human Ig framework one of the about three most homologous sequences from the collection.

2. A method according to Claim 1, wherein the human Ig sequence is selected from a collection of at least about ten to twenty Ig chain sequences.

3. A method according to Claim 1, wherein the human Ig chain sequence selected has the highest homology in the collection to the donor Ig sequence.

4. A method according to Claim 1, wherein the human Ig framework sequence selected is at least about 65% homologous to the donor Ig framework sequence.

5. A method according to Claim 1, wherein the immunoglobulin chain is a heavy chain.

6. A method according to Claim 1, wherein the humanized Ig chain comprises a human constant region.

7. An immunoglobulin comprising two light/heavy chain pairs, wherein at least one chain is designed in accordance with Claim 1.

8. A method of designing a humanized immunoglobulin chain having a framework region from a human acceptor immunoglobulin and complementarity determining regions (CDR's) from a donor immunoglobulin capable of binding to an antigen, said method comprising the steps of substituting at least one human framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from the donor immunoglobulin at a position in the immunoglobulins where:

(a) the amino acid in the human framework region of the acceptor immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to have a side chain atom within about 3Å of the CDR's in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.

9. A method according to Claim 8, wherein the humanized immunoglobulin chain comprises in addition to the CDR's at least three amino acids from the donor immunoglobulin chosen by criteria (a), (b) or (c).

10. A method according to Claim 9, wherein at least one of the amino acids substituted from the donor is immediately adjacent a CDR.

11. A method according to Claim 9, wherein said humanized immunoglobulin chain is a heavy chain.

12. An immunoglobulin comprising two light/heavy chain pairs, wherein at least one chain is designed in accordance with Claim 8.

13. An immunoglobulin according to Claim 12, which is specifically reactive with an antigen at an affinity of at least about 10^8 M^{-1} or stronger.

14. An immunoglobulin according to Claim 12, wherein the designed chain is a light chain comprising about 214 amino acids.

15. An immunoglobulin according to Claim 12, wherein the designed chain is a heavy chain comprising about 446 amino acids.

16. A DNA sequence which upon expression encodes a humanized immunoglobulin chain according to Claim 1 or Claim 8.

17. A method for improving the affinity of a humanized immunoglobulin (Ig) to an antigen, by replacing amino acids of the human Ig framework with amino acids from the donor Ig framework at positions where:

(a) the amino acid in the human framework region of the first immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to have a side chain atom within about 3Å of the CDR's in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or the CDR's of the humanized immunoglobulin.

18. A method according to Claim 17, wherein the additional amino acids comprise up to three amino acids, each of which is immediately adjacent to one of the CDR's in the second Ig.

19. A method according to Claim 17, wherein the additional amino acids comprise one amino acid immediately adjacent to a CDR.

5 20. A method according to Claim 17, wherein the additional amino acids comprise at least two amino acids from the donor Ig which are predicted by modelling to be capable of interacting with the antigen or the CDR's.

10 21. A method according to Claim 20, wherein said two or more amino acids are predicted to be within about 3A of the donor Ig CDR's.

15 22. A method according to Claim 17, wherein the humanized Ig has an affinity to the antigen within about 2 to 3 fold of the donor Ig.

23. A method according to Claim 17, wherein the antigen is a protein.

20 24. A method of producing a humanized immunoglobulin containing a heavy chain and a light chain designed in accordance with Claim 17, said method comprising:
25 culturing a host capable of expressing said heavy chain, said light chain, or both, under conditions suitable for production of said chains; and
recovering from the culture said humanized immunoglobulin.

30 25. A polynucleotide composition comprising a DNA sequence coding for a humanized immunoglobulin designed in accordance with Claim 17.

35 26. A method of producing an improved humanized immunoglobulin comprising expressing the polynucleotide composition of Claim 25.

27. A cell transformed with a polynucleotide composition according to Claim 25.

28. A composition comprising a humanized immunoglobulin secreted by a cell line according to Claim 24.

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DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

ABSTRACT OF THE DISCLOSURE

5 Novel methods for designing humanized
immunoglobulins having one or more complementarity
determining regions (CDR's) from a donor immunoglobulin and a
framework region from a human immunoglobulin comprising first
10 comparing the framework or variable region amino acid
sequence of the donor immunoglobulin to corresponding
sequences in a collection of human immunoglobulin chains, and
selecting as the human immunoglobulin one of the more
homologous sequences from the collection. Each humanized
immunoglobulin chain may comprise about 3 or more amino acids
15 from the donor immunoglobulin in addition to the CDR's,
usually at least one of which is immediately adjacent to a
CDR in the donor immunoglobulin. The heavy and light chains
may each be designed by using any one or all three additional
position criteria. When combined into an intact antibody,
20 the humanized immunoglobulins of the present invention will
be substantially non-immunogenic in humans and retain
substantially the same affinity as the donor immunoglobulin
to the antigen, such as a protein or other compound
containing an epitope.
25

FIGURE 1

1	Q	V	Q	L	Q	Q	S	G	A	E	L	A	K	P	G	A	S	V	K	M	
1	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V	
21	S	C	K	A	S	G	Y	T	F	T	S	Y	R	M	H	W	V	K	Q	R	
21	S	C	K	A	S	G	G	T	F	S	R	S	A	I	I	W	V	R	Q	A	
							*			*	-----										
41	P	G	Q	G	L	E	W	I	G	Y	I	N	P	S	T	G	Y	T	E	Y	
41	P	G	Q	G	L	E	W	M	G	G	I	V	P	M	F	G	P	P	N	Y	
							*	-----													
61	N	Q	K	F	K	D	K	A	T	L	T	A	D	K	S	S	S	T	A	Y	
61	A	Q	K	F	Q	G	R	V	T	I	T	A	D	E	S	T	N	T	A	Y	
	-----						*	*													
81	M	Q	L	S	S	L	T	F	E	D	S	A	V	Y	Y	C	A	R	G		
81	M	E	L	S	S	L	R	S	E	D	T	A	F	Y	F	C	A	G	G	Y	
												*		*			*	-----			
100	G	G	V	F	D	Y	W	G	Q	G	T	T	L	T	V	S	S				
101	G	I	Y	S	P	E	E	Y	N	G	G	L	V	T	V	S	S				
	-----						*	*	*	*											

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[illegible]

FIGURE 3

10 20 30 40 50 60
TCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTGCACT
M G W S W I F L F L L S G T A G V H

70 80 90 100 110 120
CTCAGGTCCAGCTTGTCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTGAAGG
S Q V Q L V Q S G A E V K K P G S S V K

130 140 150 160 170 180
TCTCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACAGGATGCACTGGGTAAGGCAGG
V S C K A S G Y T F T S Y R M H W V R Q

190 200 210 220 230 240
CCCCTGGACAGGGTCTGGAATGGATTGGATATATTAATCCGTCGACTGGGTATACTGAAT
A P G Q G L E W I G Y I N P S T G Y T E

250 260 270 280 290 300
ACAATCAGAAGTTCAAGGACAAGGCAACAATTACTGCAGACGAATCCACCAATACAGCCT
Y N Q K F K D K A T I T A D E S T N T A

310 320 330 340 350 360
ACATGGAAGTGAAGCAGCCTGAGATCTGAGGACACCGCAGTCTATTACTGTGCAAGAGGGG
Y M E L S S L R S E D T A V Y Y C A R G

370 380 390 400 410 420
GGGGGGTCTTTGACTACTGGGGCCAAGGAACCCTGGTCACAGTCTCCTCAGGTGAGTCCT
G G V F D Y W G Q G T L V T V S S

430
TAAAACCTCTAGA

FIGURE 4

10 20 30 40 50 60
TCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGATCAA
M E T D T L L L W V L L L W V P G S

70 80 90 100 110 120
CCGGAGATATTGAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGATAGGG
T G D I Q M T Q S P S T L S A S V G D R

130 140 150 160 170 180
TCACCATAACCTGCTCTGCCAGCTCAAGTATAAGTTACATGCACTGGTACCAGCAGAAGC
V T I T C S A S S S I S Y M H W Y Q Q K

190 200 210 220 230 240
CAGGCAAAGCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTG
P G K A P K L L I Y T T S N L A S G V P

250 260 270 280 290 300
CTCGCTTCAGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGC
A R F S G S G S G T E F T L T I S S L Q

310 320 330 340 350 360
CAGATGATTTGCGCACTTATTACTGCCATCAAAGGAGTACTTACCCACTCACGTTCCGGTC
P D D F A T Y Y C H Q R S T Y P L T F G

370 380 390 400
AGGGGACCAAGGTGGAGGTCAAACGTAAGTACACTTTTCTAGA
Q G T K V E V K

FIGURE 5

A

HES12 AGCTTCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTG
CACTCTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTG
AAGGTC

HES13 CCCAGTCGACGGATTAATATATCCAATCCATTCCAGACCCTGTCCAGGGGCCTGCCTTAC
CCAGTGCATCCTGTAGCTAGTAAAGGTGTAGCCAGAAGCCTTGCAGGAGACCTTCACGCT
CGAGCCAGG

HES14 TATATTAATCCGTCGACTGGGTATACTGAATACAATCAGAAGTTCAAGGACAAGGCAACA
ATTACTGCAGACGAATCCACCAATACAGCCTACATGGAAGTGAAGCAGCCTGAGATCTGAG
GACA

HES15 ATATCGTCTAGAGGTTTTAAGGACTCACCTGAGGAGACTGTGACCAGGGTTCCTTGGCCC
CAGTAGTCAAAGACCCCCCCCCCTCTGCACAGTAATAGACTGCCGGTGTCTCAGATCTC
AGGCTGCT

B

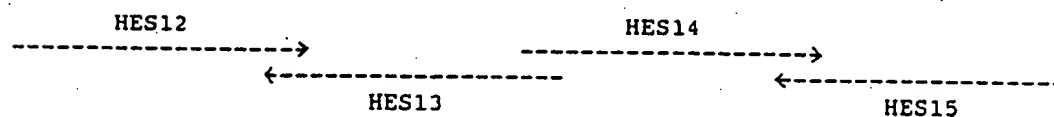


FIGURE 6

A

JFD1 CAAATCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGA
TCAACCGGAGATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGAT

JFD2 ATAAATTAGAAGCTTGGGAGCTTTGCCTGGCTTCTGCTGGTACCAGTGCATGTAATTAT
ACTTGAGCTGGCAGAGCAGGTTATGGTGACCCTATCCCCGACGCTAGCAGAGAG

JFD3 GCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTC
AGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGCCAGATGAT
TTC

JFD4 TATATCTAGAAAAGTGTACTTACGTTTGACCTCCACCTTGGTCCCCTGACCGAACGTGAG
TGGGTAAGTACTCCTTTGATGGCAGTAATAAGTGGCGAAATCATCTGGCTGCAGAGAGCT
GA

B

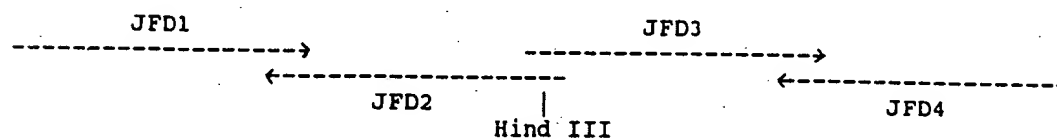


FIGURE 7

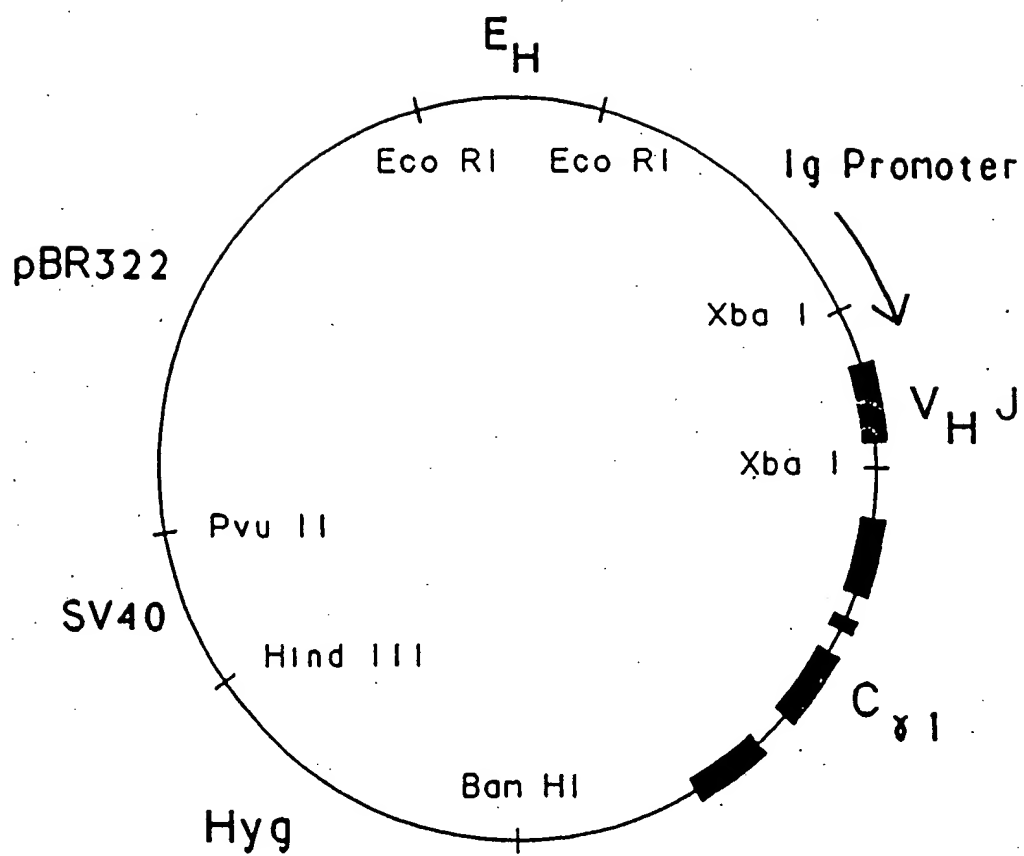


FIGURE 8

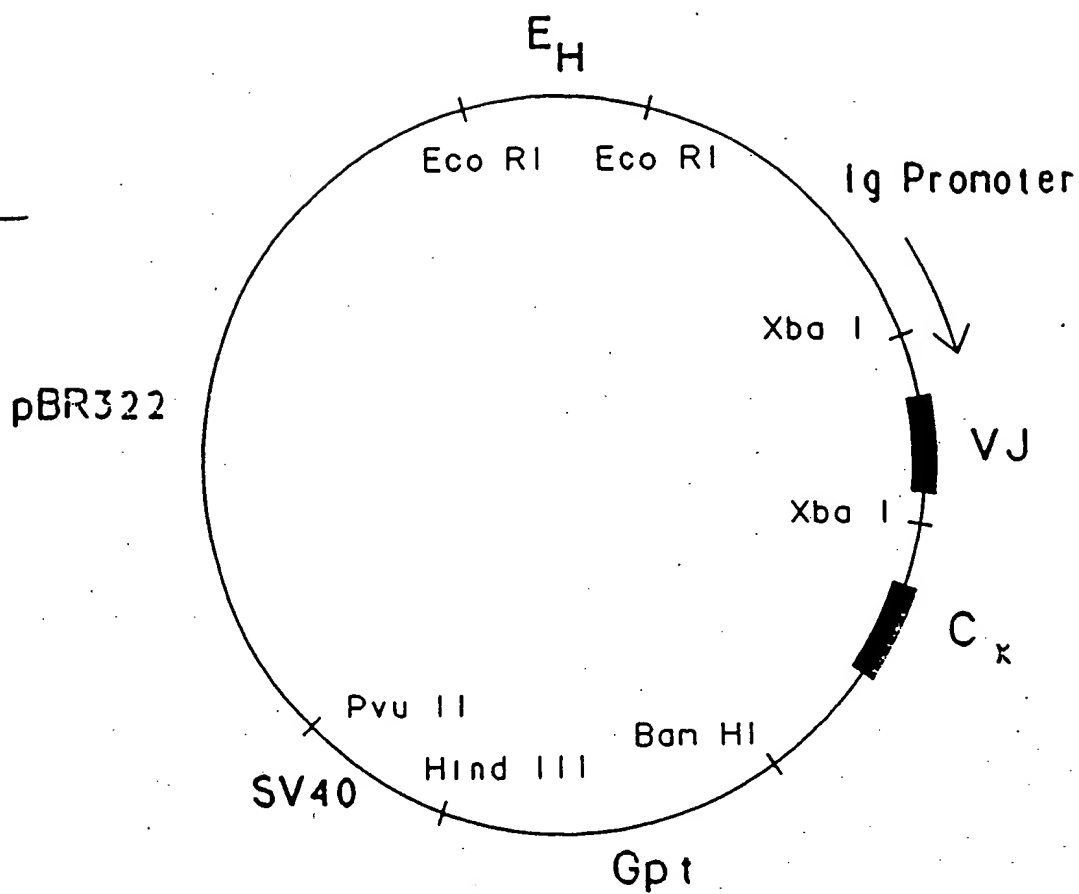


FIGURE 9

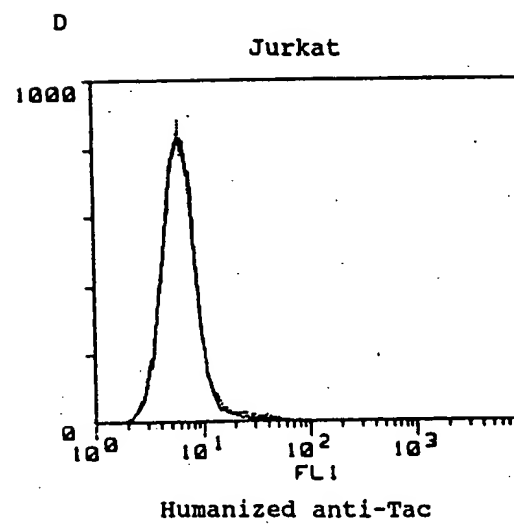
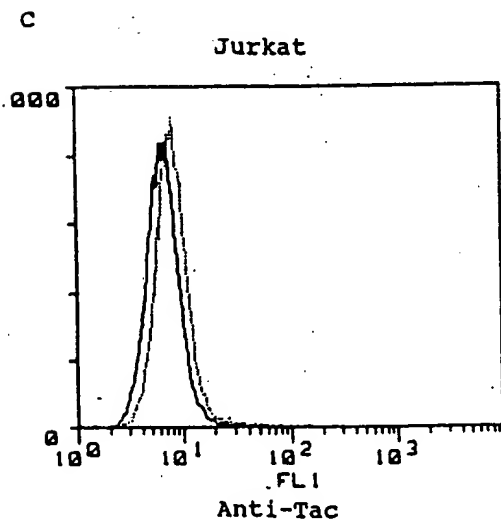
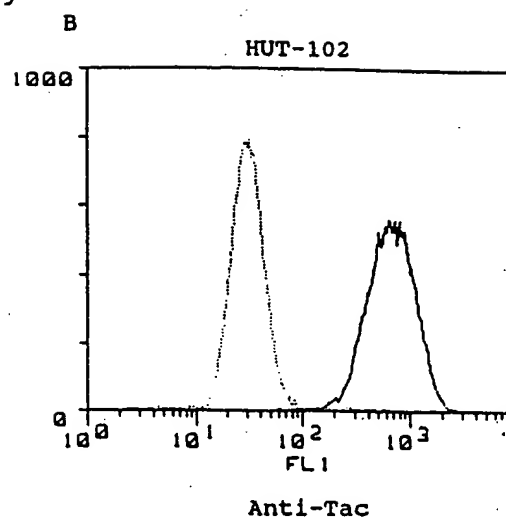
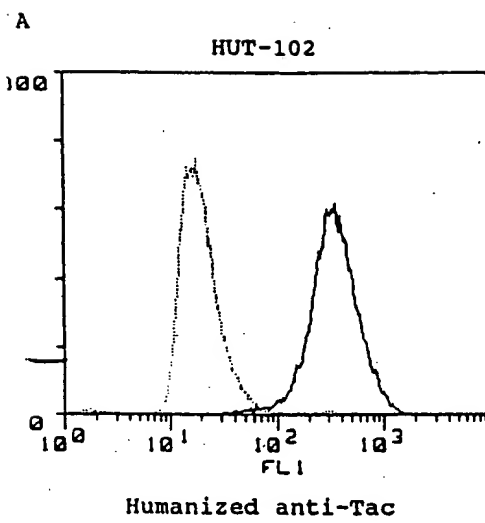
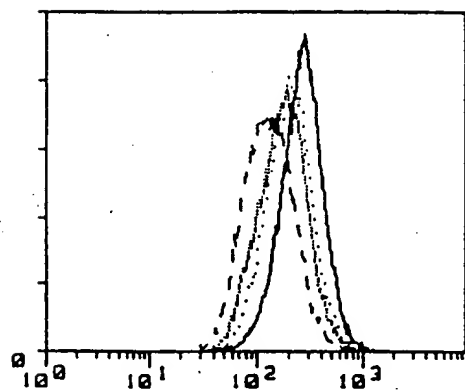


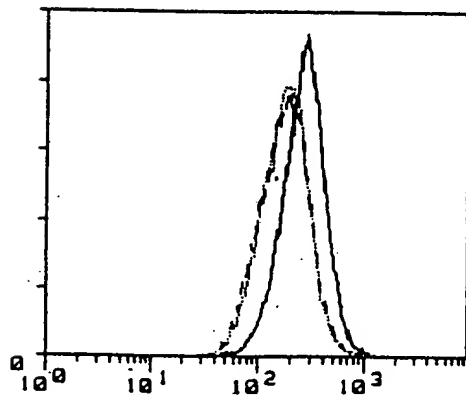
FIGURE 10

A



— 0 ng anti-Tac
..... 10 ng
----- 20 ng
- . - . - 40 ng

B



— 0 ng anti-Tac
..... 20 ng anti-Tac
----- 20 ng humanized anti-Tac



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

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SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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07/634,278	12/19/90	QUEEN	
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C 11823-26

EXAMINER

FEISEE, L

ART UNIT

PAPER NUMBER

TOWNSEND & TOWNSEND
STEUART STREET TOWER
ONE MARKET PLAZA
SAN FRANCISCO, CA 94105

1806
DATE MAILED:

7

05/07/91

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 11/25/91 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- | | |
|--|---|
| <input checked="" type="checkbox"/> Notice of References Cited by Examiner, PTO-892. | 2. <input type="checkbox"/> Notice re Patent Drawing, PTO-948. |
| <input checked="" type="checkbox"/> Notice of Art Cited by Applicant, PTO-1449. <i>Applicant</i> | 4. <input type="checkbox"/> Notice of Informal Patent Application, Form PTO-152 |
| <input type="checkbox"/> Information on How to Effect Drawing Changes, PTO-1474. | 6. <input type="checkbox"/> |

Part II SUMMARY OF ACTION

1. ☒ Claims 1-84 are pending in the application.

Of the above, claims 1-15, 24-82, 84, 85 are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☒ Claims 86-94, claims 16-23, 83 previously rejected are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☐ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____, has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).

12. ☐ Acknowledgement is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.

13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☒ Other

*claims 16-23, 83 rejected as per
Previous Office Action paper #4*

EXAMINER'S ACTION

Serial No. 634278

Art Unit 1806

This is a supplementary action to the Office action filed on 11/27/91. Due to the crossing of papers in the mail, it was necessary to reexamine the claims that were submitted in paper #6. The prior art of Uchiyama will not be submitted with this action as it was previously submitted in attachment to paper #4.

Claims 86-94 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject, matter which applicant regards as the invention. Claim 86 is indefinite in the use of the language "at least about" in that it is not clear what are the metes and bounds of the invention. Is the affinity 10' or 10" or a higher affinity? Claim 86 is further indefinite in that the parenthetical expression (CDR) is redundant and it is suggested that consistent terminology be used throughout the text of the claims. Claim 86 is indefinite in the use of the language "capable of blocking the binding" as it is unclear under what conditions this blocking effect occurs. Claim 86 is vague in the use of the language "different", in that the nature of this difference is unclear. ie. a different source, a different portion of the antibody etc.. Claim 92 is indefinite in that figures 1A and B have two light chains and two heavy chains (one is mouse anti-tac and the other is human EU), it is not clear which amino acids are indicated in the figure.

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The following is a quotation of the first paragraph of 35
U.S.C. § 112:

5 The specification shall contain a written
description of the invention, and of the manner and
process of making and using it, in such full, clear,
concise, and exact terms as to enable any person
skilled in the art to which it pertains, or with which
it is most nearly connected, to make and use the same
10 and shall set forth the best mode contemplated by the
inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112,
first paragraph, as failing to adequately teach how to make or
use the claimed invention ie. failing to provide an enabling
disclosure. The following recitations lack support in the
15 disclosure :

1) The language "at least one residue immediately adjacent
to at least one of said CDRs are from different immunoglobulin
molecules than the framework regions". It is unclear that the
different amino acid residues other either side of the CDRs would
20 behave as that which is disclosed in the specification.

As the claims read the invention can comprise a wide variety of
immunoglobulins. If at least one residue immediately adjacent to
one of the residues can be different, ie. mutated than the scope
of the claims in its current form is that all of the residues
25 adjacent to the CDR can be "different" or mutated. Applicant has
failed to show that any one of the known twenty amino acids can
be placed in the region immediately adjacent to at least one of
the CDRs and result in the antibody which is being claimed.

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Furthermore, applicant has failed to show that these residue changes can occur adjacent to two or three or sometimes in all four of the CDRs and still maintain the function which is attributed to the antibody by the disclosure. As applicant is aware, the primary structure of a protein which is determined by its amino acid composition is important to the conformation and therefore, the function of said protein. Should a hydrophilic amino acid be substituted for a hydrophobic amino acid in a crucial location of the antibody, the entire conformation of the antibody can be altered. Applicant has not shown exactly which, how many and in which location the amino acid may be "different", and has therefore failed to show how to make the invention as claimed. It also appears from the specification that the framework regions and the CDRs should be unusually homologous or should be comprised of a consensus sequence which is found in many human antibodies. This requirement also provides a degree of unpredictability in determining the "likeness" of the "donor" and the "acceptor" immunoglobulins, as they are called.

2) The language "has been modified so as to retain substantially the same antigen binding function of said different antibody".

Applicant has failed to describe what he considers to be "substantially the same", i.e. the same binding affinity, the same epitope specificity, the same effector function (binding function

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does not necessarily mean antigen binding). He has further failed to show that an antibody which has substantially the same binding function will behave as that antibody which is being claimed. Furthermore, applicant claims that said antibody be
5 modified so that it has "substantially" the same antigen binding function. Firstly, it is not clear what is meant by modified, ie. conjugated, mutated, truncated, glycosylated, aglycosylated, chimerized, amino acid substitutions, etc. Secondly, applicant has not proven that any or all of these modifications would lead
10 to functional antibodies. Conjugation is well known in the art, mutating proteins is also practiced, although with little predictability, because the primary structure of an antibody is important to its function, and in many cases even a distant change in amino acid composition will alter the binding affinity
15 or even binding ability. Glycosylation and aglycosylation lead to different affinities and sometimes to lack of antigen recognition or even effector function. Applicant has not provided enough guidance in the specification and the state of the art is not at a such an advanced state so as to provide
20 parameters for preparing these "modified" antibodies and maintaing the functions and affinities which are claimed and disclosed without undue experimentation.

3) Another one of the embodiments of the instant invention is that an antibody bind to the IL-2 receptor and block the

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binding of IL-2 to human IL-2 receptors. Obviously not all antibodies which bind to IL-2 receptors are capable of blocking binding to IL-2, and this function is reserved for antibodies which bind to that specific epitope of IL-2 receptor which binds
5 to its ligand. Applicant has only exemplified one antibody which is capable of doing this as being the anti-tac antibody which binds to an epitope of p55 Tac protein. One of skill in the art would not find the success of one antibody manipulated as that which is disclosed, sufficient basis to extrapolate to any and
10 all antibodies which bind to IL-2 receptor for the same reasons as discussed in the previous paragraphs. One of skill in the art would be forced into undue experimentation in order to make the myriad antibodies that are being claimed in view of the lack of sufficient guidance provided in the specification for the reasons
15 previously discussed.

4) Claims 92-94 are drawn to an anti-IL2 receptor antibody which consists essentially of a particular number of residues and a framework region, but it is doubtful that as written the claimed invention would work because it is not clear whether
20 these amino acids that have been listed are the only ones which are necessary for binding to the desired epitope, furthermore, it is unclear where in relation to these amino acids the framework region is located. It is suggested that a contiguous amino acid sequence be listed to indicated the antibody which is being

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claimed or a deposit of a cell line which produces said antibody be made under appropriate requirements of the deposit rules (see MPEP 608.01(p)).

Claims 86-94 are rejected under 35 U.S.C. § 112, first
5 paragraph, for the reasons set forth in the objection to the specification.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

10 A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences
15 between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

20 Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject
25 matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 86-91 are rejected under 35 U.S.C. § 103 as being unpatentable over Reichmann et. al. in view of Uchiyama et. al. or Diamantstein and further in view of Chothia et. al.. The
30 above claims are drawn to an immunoglobulin, having a specific affinity that binds to human IL-2 receptor which has CDRs from a "different" immunoglobulin molecule than its framework region and

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at least one residue immediately adjacent to the CDR which is different from the framework region. Reichmann et. al. describe the production of an antibody (HuVHCAMP) which binds to CAMPATH, they further teach the mutation of specific amino acids in the
5 framework region and state "that alterations in the 'Kabat' framework region can enhance the affinity of the antibody and extends previous work in which an engineered change in the hypervariable region yielded an antibody with increased affinity" (page 326). Both Uchiyama et. al. and Diamantstein teach
10 monoclonal antibodies which recognize human IL-2 receptor (anti-tac antibodies) and further suggest that these antibodies may be used for therapeutic and diagnostic purposes. Diamantstein even suggests that these antibodies may be made into chimeric antibodies to use in vivo. Chothia et. al. analyze the
15 hypervariable regions of various antibodies and suggest the different amino acids which may be changed in conserved and non-conserved regions, and further describe the implication of these changes. It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to
20 make humanized anti-tac antibodies according to the procedure of Reichmann et. al. using the antibodies of Diamantstein et. al. or Uchiyama et. al. in view of the potential utility of the antibody in treating T cell mediated disorders as is suggested in the Diamantstein application (see claims). One of ordinary skill

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would have had within his/her means all of the starting material and all of the information necessary to make the necessary mutations given the teachings of Chothia et. al. which describe the canonical structures of the hypervariable regions of
5 immunoglobulin (see entire document).

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. § 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered
10 therein were made absent any evidence to the contrary. Applicant is advised of the obligations under 37 C.F.R. 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential
15 U.S.C. § 102(f) or (g) prior art under 35 U.S.C. § 103.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lila Feisee whose telephone number is (703) 308-2731.

Any inquiry of a general nature or relating to the status of
20 this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO FAX Center located in Crystal Mall 1. The faxing

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of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 FAX Center number is (703) 308-4227. The hours of operation of the Center are 8:45 am - 4:45 pm, Monday - Friday.

5 Feisee/lf
April 22, 1992


JOHN J. DOLL
SUPERVISORY PATENT EXAMINER
GROUP 180

LIST OF REFERENCES
CITED BY APPLICANT

Docket No: 11823-26 Serial No: 07/634,278
Applicant(s): CARY L. QUEEN ET AL. Group No: 185
Filing Date: December 19, 1990 Page No: 1 of 4

U.S. PATENT DOCUMENTS

Examiner Initial	No.	Document No.	Date	Name	Class	Subclass	File Date If Approp.
<i>CB</i>	AA	4,816,397	3/28/89	Boss et al.		435/48	
<i>CB</i>	AB	4,816,567	3/28/89	Cabilly et al.		530/387	

FOREIGN PATENT DOCUMENTS

Examiner Initial	No.	Document No.	Date	Country	Class/Translation Subclass	Yes	No
<i>CB</i>	AC	0,239,400	9/30/87	EPO			
<i>CB</i>	AD	0,173,494	3/5/86	EPO			
<i>CB</i>	AE	0,184,187	6/11/86	EPO			
<i>CB</i>	AF	WO89/01783	3/9/89	PCT			
<i>CB</i>	AG	WO86/05513	9/25/86	PCT			
<i>CB</i>	AH	WO87/02671	5/7/87	PCT			
<i>CB</i>	AI	0,171,496	2/19/86	EPO			
<i>CB</i>	AJ	0,266,663	6/11/88	EPO			
<i>CB</i>	AK	2,188,941	10/14/87	GB			

OTHER REFERENCES

Examiner Initial	No.	Author, Title, Date, Pertinent Pages, Etc.
<i>CB</i>	A1	Vitteta et al., "Redesigning Nature's Poisons to Create Anti-Tumor Reagents," Science 238:1098-1104 (1987).
<i>CB</i>	A2	Ellison et al., "The nucleotide sequence of a human immunoglobulin C(gamma) ₁ gene", Nucleic Acids Res. 10:4071- (1982).

EXAMINER <i>[Signature]</i>	DATE CONSIDERED 4/22/92
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LIST OF REFERENCES
CITED BY APPLICANT

Docket No: 11823-26 Serial No: 07/634,278
Applicant(s): CARY L. QUEEN ET AL. Group No: 185
Filing Date: December 19, 1990 Page No: 2 of 4

U.S. PATENT DOCUMENTS

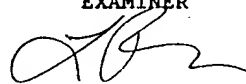
Examiner Initial	No.	Document No.	Date	Name	Class	Subclass	File Date If Approp.
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FOREIGN PATENT DOCUMENTS

Examiner Initial	No.	Document No.	Date	Country	Class/Translation Subclass	Yes	No
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OTHER REFERENCES

Examiner Initial	No.	Author, Title, Date, Pertinent Pages, Etc.
<u>VB</u>	A3	Hieter et al., "Cloned Human and Mouse Kappa Immunoglobulin Constant and J Region Genes Conserve Homology in Functional Segments", <u>Cell</u> 22:197-207 (1980).
<u>VB</u>	A4	Sharon et al., "Expression of a V _H chimaeric protein in mouse myeloma cells", <u>Nature</u> 309:364-367 (1984).
<u>VB</u>	A5	Takeda et al., "Construction of chimaeric processed immunoglobulin genes containing mouse variable and human constant region sequences", <u>Nature</u> 314:452-454 (1985).
<u>VB</u>	A6	Tan et al., "A Human-Mouse Chimeric Immunoglobulin Gene with a Human Variable Region is Expressed in Mouse Myeloma Cells", <u>J. Immunol.</u> 135:3564-3567 (1985).
<u>VB</u>	A7	Morrison et al., "Chimeric human antibody molecules: Mouse antigen-binding domains with human constant region domains," <u>Proc. Natl. Acad. Sci.</u> 81:6851-6859 (1984).
<u>VB</u>	A8	Boulianne et al., "Production of functional chimeric mouse/human antibody," <u>Nature</u> 312:643-646 (1984).
<u>VB</u>	A9	Neuberger et al., "A hapten-specific chimeric IgE antibody with human physiological effector function," <u>Nature</u> 314:268-270 (1985).
<u>VB</u>	A10	Morrison, S.L., "Transfectomas Provide Novel Chimeric Antibodies," <u>Science</u> 229:1202-1207 (1985).

EXAMINER 	DATE CONSIDERED 4/22/92
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**LIST OF REFERENCES
CITED BY APPLICANT**

Docket No: 11823-26 Serial No: 07/634,278
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FOREIGN PATENT DOCUMENTS

Examiner Initial	No.	Document No.	Date	Country	Class/Translation Subclass Yes No
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OTHER REFERENCES

Examiner Initial	No.	Author, Title, Date, Pertinent Pages, Etc.
<i>LO</i>	A11	Sahagan et al., "A Genetically Engineered Murine/Human Chimeric Antibody Retains Specificity for Human Tumor-Associated Antigen", <u>J. Immunol.</u> 137:1066-1074 (1986).
<i>LO</i>	A12	Liu et al., "Expression of mouse::human immunoglobulin heavy-chain cDNA in lymphoid cells", <u>Gene</u> 54:33-40 (1987).
<i>LO</i>	A13	Better et al., "Escherichia coli Secretion of an Active Chimeric Antibody Fragment", <u>Science</u> 240:1041-1043 (1988).
<i>LO</i>	A14	Waldmann, T.A., "The Structure, Function, and Expression of Interleukin-2 Receptors on Normal and Malignant Lymphocytes," <u>Science</u> 232:727-732 (1986).
<i>LO</i>	A15	Leonard et al., "The human receptor for T-cell growth factor," <u>J. Biol. Chem.</u> 260:1872-1880 (1985).
<i>LO</i>	A16	Uchiyama et al., "A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T-cells," <u>J. Immunol.</u> 126:1393-1397 (1981).
<i>LO</i>	A17	Farrar, J., "The biochemistry, biology, and role of interleukin-2 in the induction of cytotoxic T cell and antibody-forming B cell receptors," <u>Immunol. Rev.</u> 63:129-166 (1982).
<i>LO</i>	A18	Greene et al., "Growth of Human T Lymphocytes: An Analysis of Interleukin 2 and Its Cellular receptor", in <u>Progress in Hematology XIV</u> , E. Brown, ed., Grune and Statton, New York (1986) p.283-301.

duplicate

EXAMINER <i>[Signature]</i>	DATE CONSIDERED 4/22/92
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LIST OF REFERENCES
CITED BY APPLICANT

Docket No: 11823-26 Serial No: 07/634,278
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U.S. PATENT DOCUMENTS

Examiner Initial	No.	Document No.	Date	Name	Class	Subclass	File Date If Approp.
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FOREIGN PATENT DOCUMENTS

Examiner Initial	No.	Document No.	Date	Country	Class/Translation Subclass	Yes	No
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OTHER REFERENCES

Examiner Initial	No.	Author, Title, Date, Pertinent Pages, Etc.
<u>AB</u>	A19	Verhoyen et al., "Reshaping Human Antibodies: Grafting an Antilysozyme Activity", <u>Science</u> 239:1534-1536 (1988).
<u>AB</u>	A20	Jones et al., "Replacing the complementarity-determining regions in a human antibody with those from a mouse", <u>Nature</u> 321:522-525 (1986).
<u>AB</u>	A21	Hale et al., "Remission Induction in Non-Hodgkin Lymphoma with Reshaped Human Monoclonal Antibody CAMPATH-1H", <u>Lancet</u> December 17, 1988, pp. 1394-1399.
<u>AB</u>	A22	Chothia, C. and A.M. Lesk, "Canonical Structures for the Hypervariable Regions of Immunoglobulins", <u>J. Mol. Biol.</u> 196:901-917 (1987).
<u>AB</u>	A23	Reichmann et al., "Reshaping human antibodies for therapy", <u>Nature</u> 332:323-327 (1988).
<u>AB</u>	A24	Bird et al., "Single-Chain Antigen-Binding Proteins", <u>Science</u> 242:423-426 (1988).
<u>AB</u>	A25	Huston et al., "Protein engineering of antibody binding sites: Recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in <i>Escherichia coli</i> ", <u>Proc. Natl. Acad. Sci. U.S.A.</u> 85:5879-5883 (1988).in <u>Progress in Hematology XIV</u> , E. Brown, ed., Grune and Statton, New York (1986) p.283.

EXAMINER <u>AB</u>	DATE CONSIDERED 4/22/92
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FORM PTO-1449 (Modified)				Attorney Docket No. 11823-26		Serial No. 07/634,278	
LIST OF PATENTS AND PUBLICATIONS FOR APPLICANT'S INFORMATION DISCLOSURE STATEMENT				Applicant CARY L. QUEEN ET AL.			
				Filing Date 12/19/90		Group 1806	
REFERENCE DESIGNATION* U.S. PATENT DOCUMENTS							
Examiner Initial	*	Document No.	Date	Name		Class	Sub- Class
AA	5	2	2	5	5	3	9
			07/06/93	GREGORY P. WINTER		530	370.3
AB							
FOREIGN PATENT DOCUMENTS							
Examiner Initial	*	Document No.	Date	Country		Trans- lation?	Sub- Class
AC							
OTHER ART (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)							
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By: Linda K. Johnston

PATENT
11823-26

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of)
CARY L. QUEEN ET AL.) Examiner: L. Faisee
Serial No.: 07/634,278) Art Unit: 185
Filed: December 19, 1990) PRELIMINARY AMENDMENT
For: HUMANIZED IMMUNOGLOBULINS)
San Francisco, CA 94105

Commissioner of Patents and Trademarks
Washington, D. C. 20231

Sir:

In The Claims

Please add the following new claims:

86. An immunoglobulin having two pairs of chain/heavy chain dimers which binds specifically to an epitope of the human interleukin-2 receptor with an affinity of at least about $10^6 M^{-1}$, said light and heavy chains comprising complement determining regions (CDR's) and human framework regions, wherein the CDR's and at least one residue immediately adjacent to at least one of said CDRs are from different immunoglobulin molecules than the framework regions, and wherein said human framework region (a) is identical to a native human framework region, or (b) has been modified so as to retain the substantially the same antigen binding function of said different antibody.

87. An immunoglobulin according to Claim 86, which binds to an epitope located on a p55 Tac protein.

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US 14027 12/19/91 07634278

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20-1430 140 103

88. An immunoglobulin according to Claim 86, which is capable of blocking the binding of interleukin-2 (IL-2) to human IL-2 receptors.

89. An immunoglobulin according to Claim 86, wherein the human framework regions comprise amino acids sequences from at least two human immunoglobulins.

90. An immunoglobulin according to Claim 86, wherein the CDR's are from a mouse immunoglobulin.

91. An immunoglobulin according to Claim 86 which was produced in a myeloma or hybridoma cell.

92. An immunoglobulin having two pairs of heavy and light chains which binds specifically to the human interleukin-2 receptor, wherein a) the heavy chain consists essentially of residues 27, 37, 31-35, 50-66, 93, 95, 98, 99-106, 107-109, and 111 of the human heavy chain amino acid sequence of Figure 1B and a human framework region; and b) the light chain consists essentially of residues 24-34, 48, 50-56, 60, 63 and 89-97 of the light chain amino acid sequence of Figure 1A and a human framework region, wherein said human framework region (i) is identical to a native human framework region; or (ii) has been modified so as to retain substantially the same antigen binding function of said different antibody.

93. An immunoglobulin of claim 92, wherein the human framework region of the heavy chain is the EU heavy chain framework region of Figure 1B.

94. An immunoglobulin of claim 92, wherein the human framework region of the light chain is the EU light chain framework region of Figure 1A.

REMARKS

Claims 86-94, which are directed to the same general invention as claims 16-23, were allowed in parent application U.S.S.N. 07/290,975 (with appropriate figure references). However, the issue fee was not paid and, thus, the allowed claims are resubmitted here in accordance with In re Bartfeld, 17 USPQ 2nd 1885 (CAFC 1991).

Respectfully submitted,

TOWNSEND and TOWNSEND

Date: 11-25-91

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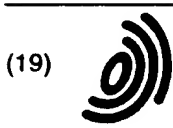
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(54) HUMANIZED IMMUNOGLOBULINS AND THEIR PRODUCTION AND USE
HUMANISIERTE IMMUNGLOBULINE, DEREN HERSTELLUNG UND VERWENDUNG
IMMUNOGLOBULINES HUMANISES, LEURS PRODUCTION ET UTILIZATION

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(56) References cited:
EP-A- 0 239 400 WO-A-89/01783
WO-A-89/09622 GB-A- 2 188 941
US-A- 4 816 567

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- SCIENCE, vol. 238, 20 November 1987; VITETTA et al., pp. 1098-1104/
- SCIENCE, vol. 229, 20 September 1985; MORRISON, pp. 1202-1207/
- SCIENCE, vol. 232, 09 May 1986; WALDMANN, pp. 727-732/
- JOURNAL OF IMMUNOLOGY, vol. 126, no. 4, 04 April 1981; UCHIYAMA/
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- NATURE, vol. 321, 29 May 1986; JONES et al., pp. 522-525/
- NATURE, vol. 332, 24 March 1988; RIECHMANN et al., pp. 323-326/

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

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Description**Field of the Invention**

5 The present invention relates generally to the combination of recombinant DNA and monoclonal antibody technologies for developing novel therapeutic agents and, more particularly, to the production of non-immunogenic antibodies and their uses.

Background of the Invention

10 In mammals, the immune response is mediated by two types of cells that interact specifically with foreign material, i.e., antigens. One of these cell types, B-cells, are responsible for the production of antibodies. The second cell class, T-cells, include a wide variety of cellular subsets controlling the in vivo function of both B-cells and a wide variety of other hematopoietic cells, including T-cells.

15 One way in which T-cells exert this control is through the production of a lymphokine known as interleukin-2 (IL-2), originally named T-cell growth factor. IL-2's prime function appears to be the stimulation and maintenance of T-cells. Indeed, some immunologists believe that IL-2 may be at the center of the entire immune response (see, Farrar, J., et al., Immunol. Rev. 63:129-166 (1982)).

20 To exert its biological effects, IL-2 interacts with a specific high-affinity membrane receptor (Greene, W., et al., Progress in Hematology XIV, Brown, Ed., Grune and Statton, New York (1986), at pgs. 283 ff). The human IL-2 receptor is a complex multichain glycoprotein, with one chain, known as the Tac peptide, being about 55kD in size (see, Leonard, W., et al., J. Biol. Chem. 260:1872 (1985), which is incorporated herein by reference). A gene encoding this protein has been isolated, and predicts a 272 amino acid peptide, including a 21 amino acid signal peptide (see, Leonard, W., et al., Nature 311: 626 (1984)). The 219 NH₂-terminal amino acids of the p55 Tac protein apparently comprise an extracellular domain (see, Leonard, W., et al., Science, 230:633-639 (1985)).

25 Much of the elucidation of the human IL-2 receptor's structure and function is due to the development of specifically reactive monoclonal antibodies. In particular, one mouse monoclonal antibody, known as anti-Tac (Uchiyama, et al., J. Immunol. 126:1393 (1981)) has shown that IL-2 receptors can be detected on T-cells, but also on cells of the monocyte-macrophage family, Kupffer cells of the liver, Langerhans' cells of the skin and, of course, activated T-cells. Importantly, resting T-cells, B-cells or circulating macrophages typically do not display the IL-2 receptor (Hermann, et al., J. Exp. Med. 162:1111 (1985)).

30 The anti-Tac monoclonal antibody has also been used to define lymphocyte functions that require IL-2 interaction, and has been shown to inhibit various T-cell functions, including the generation of cytotoxic and suppressor T lymphocytes in cell culture. Also, based on studies with anti-Tac and other antibodies, a variety of disorders are now associated with improper IL-2 receptor expression by T-cells, in particular adult T-cell leukemia.

35 More recently, the IL-2 receptor has been shown to be an ideal target for novel therapeutic approaches to T-cell mediated diseases. It has been proposed that IL-2 receptor specific antibodies, such as the anti-Tac monoclonal antibody, can be used either alone or as an immunoconjugate (e.g., with Ricin A, isotopes and the like) to effectively remove cells bearing the IL-2 receptor. These agents can, for example, theoretically eliminate IL-2 receptor-expressing leukemic cells, certain B-cells, or activated T-cells involved in a disease state, yet allow the retention of mature normal T-cells and their precursors to ensure the capability of mounting a normal T-cell immune response as needed. In general, most other T-cell specific agents can destroy essentially all peripheral T-cells, which limits the agents' therapeutic efficacy. Overall, the use of appropriate monoclonal antibodies specific for the IL-2 receptor may have therapeutic utility in autoimmune diseases, organ transplantation and any unwanted response by activated T-cells. Indeed, clinical trials have been initiated using, e.g., anti-Tac antibodies (see, generally, Waldman, T., et al., Cancer Res. 45:625 (1985) and Waldman, T., Science 232:727-732 (1986)).

45 Unfortunately, the use of the anti-Tac and other non-human monoclonal antibodies have certain drawbacks, particularly in repeated therapeutic regimens as explained below. Mouse monoclonal antibodies, for example, do not fix human complement well, and lack other important immunoglobulin functional characteristics when used in humans.

50 Perhaps more importantly, anti-Tac and other non-human monoclonal antibodies contain substantial stretches of amino acid sequences that will be immunogenic when injected into a human patient. Numerous studies have shown that, after injection of a foreign antibody, the immune response elicited by a patient against an antibody can be quite strong, essentially eliminating the antibody's therapeutic utility after an initial treatment. Moreover, as increasing numbers of different mouse or other antigenic (to humans) monoclonal antibodies can be expected to be developed to treat various diseases, after the first and second treatments with any different non-human antibodies, subsequent treatments even for unrelated therapies can be ineffective or even dangerous in themselves.

55 While the production of so-called "chimeric antibodies" (e.g., mouse variable regions joined to human constant regions) (see, for example, W089/09622) has proven somewhat successful, a significant immunogenicity problem re-

mains. In general, the production of human immunoglobulins reactive with the human IL-2 receptor, as with many human antigens, has been extremely difficult using typical human monoclonal antibody production techniques. Similarly, past attempts utilizing recombinant DNA technology to produce so-called "humanized" antibodies (see e.g. EPO Publication No. 0239400), provides uncertain results, in part due to unpredictable binding affinities.

Thus, there is a need for improved forms of human-like immunoglobulins, such as those specific for the human IL-2 receptor, that are substantially non-immunogenic in humans, yet easily and economically produced in a manner suitable for therapeutic formulation and other uses. The present invention fulfills these and other needs. The hypervariable regions (also called Complementarity Determining Regions, abbreviated to "CDRs") of immunoglobulins were originally defined by Kabat et al., ("Sequences of Proteins of Immunological Interest" Kabat, E., et al., U.S. Department of Health and Human Services, (1983)) based on extent of sequence variability, to consist of residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain (V_L) and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain (V_H), using Kabat's standard numbering system for antibody amino acids. The CDRs are believed to contact the target antigen of an antibody and to be primarily responsible for binding. More recently Chothia et al (Chothia and Lesk, J. Mol. Biol., 196:901- 917 (1987)) have given an alternate definition of the hypervariable regions or CDRs as consisting of residues 26-32 (L1), 50-52 (L2), 91-96 (L3) in V_L and residues 26-32 (H1), 53- 55 (H2), 96-101 (H3) in V_H . The Chothia definition is based on the residues that constitute the loops in the 3-dimensional structures of antibodies. It is particularly important to note that for each of the six CDRs the Chothia CDR is actually a subset of (i.e. smaller than) the Kabat CDR, with the single exception of H1 (the first heavy chain CDR), where the Chothia CDR contains amino acids 26-30 that are not in the Kabat CDR.

Riechmann et al ("Reshaping human antibodies for therapy", Nature, Vol 332, pp 323-326, (March 1988)) describe work in which precisely the Kabat CDRs were transferred to a pre-determined human framework (NEW again for the heavy chain and REI for the light chain). However, they found that an antibody containing the humanized heavy chain lost most of its binding affinity and ability to lyse target cells. They therefore made a new humanized antibody containing the Kabat CDRs from the mouse antibody and two amino acid changes in Chothia CDR H1, but no other mouse amino acids.

Summary of the Invention

The invention provides the use of at least one amino acid substitution outside of complementarity determining regions (CDR's) as defined by Kabat et al ("Sequences of Proteins of Immunological Interest", Kabat, E., et al., US Department of Health and Human Services, (1983)) together with Chothia et al (Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)) in the production of a humanized immunoglobulin, wherein said at least one amino acid substitution is from the non-CDR variable region of a non-human donor immunoglobulin, and in which humanized immunoglobulin the variable region amino acid sequence other than the CDR's comprises at least 70 amino acid residues identical to an acceptor human immunoglobulin variable region amino acid sequence, and the CDR's are from the variable region of said non-human donor immunoglobulin.

In another aspect, the invention provides a method of producing a humanized immunoglobulin chain having a framework region from a human acceptor immunoglobulin and complementarity determining regions (CDR's) from a donor immunoglobulin capable of binding to an antigen, said method comprising the steps of substituting at least one non-CDR framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from the donor immunoglobulin at a position in the immunoglobulins where:

(a) the amino acid in the human framework region of the acceptor immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to have a side chain atom capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.

The present invention provides novel compositions useful, for example, in the treatment of T-cell mediated human disorders, the compositions containing human-like immunoglobulins specifically capable of blocking the binding of human IL-2 to its receptor and/or capable of binding to the p55 Tac protein on human IL-2 receptors. The immunoglobulins can have two pairs of light chain/heavy chain complexes, typically at least one pair having chains comprising mouse complementarity determining regions functionally joined to human framework region segments. For example, mouse complementarity determining regions, with or without additional naturally-associated mouse amino acid residues, can be used to produce human-like antibodies capable of binding to the human IL-2 receptor at affinity levels stronger than

about 10^8 M^{-1} .

The immunoglobulins, including binding fragments and other derivatives thereof, of the present invention may be produced readily by a variety of recombinant DNA techniques, with ultimate expression in transfected cells, preferably immortalized eukaryotic cells, such as myeloma or hybridoma cells. Polynucleotides comprising a first sequence coding for human-like immunoglobulin framework regions and a second sequence set coding for the desired immunoglobulin complementarity determining regions can be produced synthetically or by combining appropriate cDNA and genomic DNA segments.

The human-like immunoglobulins may be utilized alone in substantially pure form, or complexed with a cytotoxic agent, such as a radionuclide, a ribosomal inhibiting protein or a cytotoxic agent active at cell surfaces. All of these compounds will be particularly useful in treating T-cell mediated disorders. The human-like immunoglobulins or their complexes can be prepared in a pharmaceutically accepted dosage form, which will vary depending on the mode of administration.

Methods for designing human-like immunoglobulin chains having one or more complementarity determining regions (CDR's) from a donor immunoglobulin and a framework region from a human immunoglobulin, may involve first comparing the framework or variable region amino acid sequence of the donor immunoglobulin to corresponding sequences in a collection of human immunoglobulin chains, and selecting as the human immunoglobulin one of the more homologous sequences from the collection. The human immunoglobulin, or acceptor immunoglobulin, sequence is typically selected from a collection of at least 10 to 20 immunoglobulin chain sequences, and usually will have the highest homology to the donor immunoglobulin sequence of any sequence in the collection. The human immunoglobulin framework sequence will typically have about 65 to 70% homology or more to the donor immunoglobulin framework sequences. The donor immunoglobulin may be either a heavy chain or light chain (or both), and the human collection will contain the same kind of chain. A humanized light and heavy chain can be used to form a complete humanized immunoglobulin or antibody, having two light/heavy chain pairs, with or without partial or full-length human constant regions and other proteins.

The humanized immunoglobulin chain will typically comprise at least about 3 amino acids from the donor immunoglobulin in addition to the CDR's, usually at least one of which is immediately adjacent to a CDR in the donor immunoglobulin. The heavy and light chains may each be designed by using any one or all three of the position criteria.

When combined into an intact antibody, the humanized light and heavy chains of the present invention will be substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen (such as a protein or other compound containing an epitope). These affinity levels can vary from about 10^8 M^{-1} or higher, and may be within about 4 fold of the donor immunoglobulin's original affinity to the antigen.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Comparison of sequences of anti-Tac heavy chain (upper lines) and Eu heavy chain (lower lines). The 1-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDRs are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an *.

Figure 2. Comparison of sequences of anti-Tac light chain (upper lines) and Ed light chain (lower lines). The single-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDRs are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an *.

Figure 3. Nucleotide sequence of the gene for the humanized anti-Tac heavy chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature heavy chain sequence begins with amino acid #20 Q.

Figure 4. Nucleotide sequence of the gene for the humanized anti-Tac light chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature light chain sequence begins with amino acid #21 D.

Figure 5. A. Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac heavy chain gene, printed 5' to 3'. B. Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide.

Figure 6. (A) Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac light chain gene printed 5' to 3'. (B) Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide. The position of a Hind III site in the overlap of JFD2 and JFD3 is shown.

Figure 7. Schematic diagram of the plasmid pHuGTAC1 used to express the humanized anti-Tac heavy chain. Relevant restriction sites are shown, and coding regions of the heavy chain are displayed as boxes. The direction of transcription from the immunoglobulin (Ig) promoter is shown by an arrow. E_H = heavy chain enhancer, Hyg = hygromycin

resistance gene.

Figure 8. Schematic diagram of the plasmid pHuLTAC used to express the humanized anti-Tac light chain. Relevant restriction sites are shown, and coding regions of the light chain are displayed as boxes. The direction of transcription from the Ig promoter is shown by an arrow.

Figure 9. Fluorocytometry of HUT-102 and Jurkat cells stained with anti-Tac antibody or humanized anti-Tac antibody followed respectively by fluorescein-conjugated goat anti-mouse Ig antibody or goat anti-human Ig antibody, as labeled. In each panel, the dotted curve shows the results when the first antibody was omitted, and the solid curve the results when first and second (conjugated) antibodies were included as described.

Figure 10. (A) Fluorocytometry of HUT-102 cells stained with 0-40 ng of anti-Tac, as indicated, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin. (B) Fluorocytometry of HUT-102 cells stained with the indicated antibody, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with one embodiment of the present invention, human-like immunoglobulins specifically reactive with desired epitopes, such as those on the IL-2 receptor on human T-cells, are provided. These immunoglobulins, which have binding affinities of at least about 10^8 m^{-1} , and preferably 10^9 M^{-1} to 10^{10} M^{-1} or stronger, are capable of, e.g., blocking the binding of IL-2 to human IL-2 receptors. The human-like immunoglobulins will have a human-like framework and can have complimentary determining regions (CDR's) from an immunoglobulin, typically a mouse immunoglobulin, specifically reactive with an epitope on p55 Tac protein. The immunoglobulins of the present invention, which can be produced economically in large quantities, find use, for example, in the treatment of T-cell mediated disorders in human patients by a variety of techniques.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25kD) and one "heavy" chain (about 50-70kD). The NH_2 -terminus of each chain begins a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The C00H terminus of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as either kappa or lambda. Heavy chains are classified (and subclassified) as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 12 more amino acids. (See, generally, Fundamental Immunology, Paul, W., Ed., Chapter 7, pgs. 131- 166, Raven Press, N.Y. (1984)).

The variable regions of each light/heavy chain pair form the antibody binding site. The chains all exhibit the same general structure of relatively conserved framework regions joined by three hypervariable regions, also called CDR's (see, "Sequences of Proteins of Immunological Interest," Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)). The CDR's from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope.

As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. The immunoglobulins may exist in a variety of forms besides antibodies; including, for example, Fv, Fab, and F(ab)_2 , as well as in single chains (e.g., Huston, et al., Proc. Nat. Acad. Sci. U.S.A., 85:5879-5883 (1988) and Bird, et al., Science, 242:423-426 (1988)). (See, generally, Hood, et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, Nature, 323:15-16 (1986)).

Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin gene segments belonging to different species. For example, the variable (V) segments of the genes from a mouse monoclonal antibody may be joined to human constant (C) segments, such as γ_1 and γ_3 . A typical therapeutic chimeric antibody is thus a hybrid protein consisting of the V or antigen-binding domain from a mouse antibody and the C or effector domain from a human antibody (e.g., A.T.C.C. Accession No. CRL 9688 secretes an anti-Tac chimeric antibody), although other mammalian species may be used.

As used herein, the term "framework region" refers to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved (i.e., other than the CDR's) among different immunoglobulins in a single species, as defined by Kabat, et al., op. cit. As used herein, a "human-like framework region" is a framework region that in each existing chain comprise at least about 70 or more amino acid residues, typically 75 to 85 or more residues, identical to those in a human immunoglobulin.

As used herein, the term "human-like immunoglobulin" refers to an immunoglobulin comprising a human-like framework and in which any constant region present is substantially homologous to a human immunoglobulin constant region, i.e., at least about 85-90%, preferably about 95% identical. Hence, all parts of a human-like immunoglobulin, except possibly the CDR's, are substantially homologous to corresponding parts of one or more native human immunoglobulin

sequences. For example, a human-like immunoglobulin would not encompass a chimeric mouse variable region/human constant region antibody.

In accordance with another general aspect of the present invention, also included are criteria by which a limited number of amino acids in the framework of a human-like or humanized immunoglobulin chain are chosen to be the same as the amino acids at those positions in the donor Ig rather than in the acceptor Ig, in order to increase the affinity of an antibody comprising the humanized immunoglobulin chain.

This aspect of the present invention is based in part on the model that two contributing causes of the loss of affinity in prior means of producing humanized antibodies (using as examples mouse antibodies as the source of CDR's) are:

(1) When the mouse CDR's are combined with the human framework, the amino acids in the framework close to the CDR's become human instead of mouse. Without intending to be bound by theory, we believe that these changed amino acids may slightly distort the CDR's, because they create different electrostatic or hydrophobic forces than in the donor mouse antibody, and the distorted CDR's may not make as effective contacts with the antigen as the CDR's did in the donor antibody;

(2) Also, amino acids in the original mouse antibody that are close to, but not part of, the CDR's (*i.e.*, still part of the framework), may make contacts with the antigen that contribute to affinity. These amino acids are lost when the antibody is humanized, because all framework amino acids are made human.

To avoid these problems, and to produce humanized antibodies that have a very strong affinity for a desired antigen, the present invention uses the following four criteria for designing humanized immunoglobulins. These criteria may be used singly, or when necessary in combination, to achieve the desired affinity or other characteristics.

Criterion I: As acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. For example, comparison of the sequence of a mouse heavy (or light) chain variable region against human heavy (or light) variable regions in a data bank (for example, the National Biomedical Research Foundation Protein Identification Resource) shows that the extent of homology to different human regions varies greatly, typically from about 40% to about 60-70%. By choosing as the acceptor immunoglobulin one of the human heavy (respectively light) chain variable regions that is most homologous to the heavy (respectively light) chain variable region of the donor immunoglobulin, fewer amino acids will be changed in going from the donor immunoglobulin to the humanized immunoglobulin. Hence, and again without intending to be bound by theory, it is believed that there is a smaller chance of changing an amino acid near the CDR's that distorts their conformation. Moreover, the precise overall shape of a humanized antibody comprising the humanized immunoglobulin chain may more closely resemble the shape of the donor antibody, also reducing the chance of distorting the CDR's.

Typically, one of the 3-5 most homologous heavy chain variable region sequences in a representative collection of at least about 10 to 20 distinct human heavy chains will be chosen as acceptor to provide the heavy chain framework, and similarly for the light chain. Preferably, one of the 1-3 most homologous variable regions will be used. The selected acceptor immunoglobulin chain will most preferably have at least about 65% homology in the framework region to the donor immunoglobulin.

Regardless of how the acceptor immunoglobulin is chosen, higher affinity may be achieved by selecting a small number of amino acids in the framework of the humanized immunoglobulin chain to be the same as the amino acids at those positions in the donor rather than in the acceptor. The following criteria define what amino acids may be so selected. Preferably, at most of all amino acid positions satisfying one of these criteria, the donor amino acid will in fact be selected.

Criterion II: If an amino acid in the framework of the human acceptor immunoglobulin is unusual (*i.e.*, "rare", which as used herein indicates an amino acid occurring at that position in no more than about 10% of human heavy (respectively light) chain V region sequences in a representative data bank), and if the donor amino acid at that position is typical for human sequences (*i.e.*, "common", which as used herein indicates an amino acid occurring in at least about 25% of sequences in a representative data bank), then the donor amino acid rather than the acceptor may be selected. This criterion helps ensure that an atypical amino acid in the human framework does not disrupt the antibody structure. Moreover, by replacing an unusual amino acid with an amino acid from the donor antibody that happens to be typical for human antibodies, the humanized antibody may be made less immunogenic.

Criterion III: In the positions immediately adjacent to the 3 CDR's in the humanized immunoglobulin chain, the donor amino acid rather than acceptor amino acid may be selected. These amino acids are particularly likely to interact with the amino acids in the CDR's and, if chosen from the acceptor, distort the donor CDR's and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit *et al.*, *Science*, 233, 747-753 (1986)) and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

Criterion IV: A 3-dimensional model, typically of the original donor antibody, shows that certain amino acids outside of the CDR's are close to the CDR's and have a good probability of interacting with amino acids in the CDR's by hydrogen

bonding, Van der Waals forces, hydrophobic interactions, etc. At those amino acid positions, the donor amino acid rather than the acceptor immunoglobulin amino acid may be selected. Amino acids according to this criterion will generally have a side chain atom within about 3 angstrom units of some site in the CDR's and must contain atoms that could interact with the CDR atoms according to established chemical forces, such as those listed above. Computer programs to create models of proteins such as-antibodies are generally available and well known to those skilled in the art (see, Loew *et al.*, *Int. J. Quant. Chem., Quant. Biol. Symp.*, 15:55-66 (1988); Bruccoleri *et al.*, *Nature*, 335, 564-568 (1988); Chothia *et al.*, *Science*, 233:755-758 (1986)). These do not form part of the invention. Indeed, because all antibodies have similar structures, the known antibody structures, which are available from the Brookhaven Protein Data Bank, can be used if necessary as rough models of other antibodies. Commercially available computer programs can be used to display these models on a computer monitor, to calculate the distance between atoms, and to estimate the likelihood of different amino acids interacting (see, Ferrin *et al.*, *J. Mol. Graphics*, 6:13-27 (1988)).

Humanized or human-like antibodies generally have at least three potential advantages over mouse or in some cases chimeric antibodies for use in human therapy:

- 1) Because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement- dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).
- 2) The human immune system should not recognize the framework or constant region of the humanized antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign mouse antibody or a partially foreign dimeric antibody.
- 3) Injected mouse antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of normal antibodies (D. Shaw *et al.*, *J. Immunol.*, 138:4534-4538 (1987)). Injected humanized antibodies will presumably have a half-life more similar to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

The present invention is specifically directed to improved humanized immunoglobulins (e.g., capable of binding the human IL-2 receptor) with respect to those described in EPA publication no. 0239400. That application, the disclosure of which is excluded from coverage herein, describes, for certain immunoglobulins substituting CDR's regions in the light or heavy chain variable domains of an acceptor antibody with analogous parts of CDR's (typically solvent accessible) from an antibody of different specificity. Also, that application discusses, for certain immunoglobulins, the possibility of only transferring residues that are (solvent) accessible from the antigen binding site, which residues apparently may include certain framework regions (specifically, residues known to be involved in antigen binding as described in Amit *et al.*, *Science* 233: 747-753 (1986) or perhaps residues essential for inter-chain interactions - but for the selection of which insufficient guidance is provided in that application). Thus, for example, a preferred embodiment of the present invention entails substituting entire CDR's and framework amino acids immediately adjacent one (or preferably each) of the CDR's. In general, any framework residue that also makes contact with the CDR's to, e.g., maintain their conformation (and usually their antigen binding specificity) are specifically included within preferred embodiments of the present invention as described in detail, *supra*.

In one aspect, the present invention is directed to recombinant DNA segments encoding the heavy and/or light chain CDR's (typically with other amino acid residues as described above) from an immunoglobulin capable of binding to a desired epitope, such as on the human IL-2 receptor (e.g., the anti-Tac monoclonal antibody). The DNA segments encoding these regions will typically be joined to DNA segments encoding appropriate human-like framework regions. For example, the preferred DNA sequences, which on expression code for the polypeptide chains comprising the anti-Tac heavy and light chain hypervariable regions (with human-like framework regions), are shown in Figures 3 and 4, respectively. Due to codon degeneracy and non-critical amino-acid substitutions, other DNA sequences can be readily substituted for those sequences, as detailed below.

The DNA segments will typically further include an expression control DNA sequence operably linked to the human-like antibody coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow.

Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B-cells (see, Kabat *op. cit.* and WP87/02671). For example, the human kappa immunoglobulin constant and J region genes and sequences are described in Heiter *et al.*, *Cell* 22:197-207 (1980) and the nucleotide sequence of a human immunoglobulin C_μ gene is described in Ellison *et al.*, *Nucl. Acid. Res.* 10:4071 (1982). The CDR's for producing the immunoglobulins of the present invention will be similarly derived from

monoclonal antibodies capable of binding to the desired antigen (e.g., the human IL-2 receptor) and produced in any convenient mammalian source, including mice, rats, rabbits, or other vertebrate capable of producing antibodies by well known methods. Suitable source cells for the DNA sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A.).

In addition to the human-like immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, for the IL-2 receptor immunoglobulins the framework regions can vary from the sequences in Figures 3 and 4 at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the human-like immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Giltman and Smith, *Gen* 8:81-97 (1979) and Roberts, S. et al, *Nature* 328:731-734 (1987)). Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). Also because like many genes, the immunoglobulin-related genes contain separate functional regions, each having one or more distinct biological activities, the genes may be fused to functional regions from other genes (e.g., enzymes) to produce fusion proteins (e.g., immunotoxins) having novel properties.

The nucleic acid sequences of the present invention capable of ultimately expressing the desired human-like antibodies can be formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), as well as by a variety of different techniques. Joining appropriate genomic sequences is presently the most common method of production, but cDNA sequences may also be utilized (see, European Patent Publication No. 0239400 and Reichmann, L., et al., *Nature* 332:323-327 (1988)).

As stated previously, the DNA sequences will be expressed in hosts after the sequence have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences (see, e.g., U.S. Patent 4,704,362).

E. coli is one prokaryotic host useful particularly for cloning the DNA sequences of the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may also be used for expression. *Saccharomyces* is a preferred host, with suitable vectors having expression control sequences, such as promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (see, Winnacker, "From Genes to Clones," VCH Publishers, N.Y., N.Y. (1987)). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed in the art, and include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, etc, but preferably transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen, C., et al., *Immunol. Rev.* 89:49-68 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from SV40 with enhancer (see, Mulligan and Berg, *Science* 209:1422-1427 (1980), an immunoglobulin gene, Adenovirus, Bovine Papilloma Virus, and the like.

The vectors containing the DNA segments of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts. (See, generally, Maniatis, et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, (1982)).

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, Scopes, R., *Protein Purification*, Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95%

homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures, immunofluorescent stainings, and the like. (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The IL-2 receptor specific antibodies exemplified in the present invention will typically, find use individually in treating a T-cell mediated disease state. Generally, where the cell linked to a disease has been identified as IL-2 receptor bearing, then the human-like antibodies capable of blocking the binding of IL-2 to the human IL-2 receptor are suitable. For example, typical disease states suitable for treatment include graft versus host disease and transplant rejection in patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc. Other diseases include autoimmune diseases, such as Type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis.

The human-like antibodies of the present invention may also be used in combination with other antibodies, particularly human monoclonal antibodies reactive with other markers on cells responsible for the disease. For example, suitable T-cell markers can include those grouped into the so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Bernard, et al., Eds., Springer-Verlag, N.Y. (1984).

The antibodies can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

A preferred pharmaceutical composition of the present invention comprises the use of the subject antibodies in immunotoxins. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle," provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al, Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982).

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase, (See, generally, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985)).

The delivery component of the immunotoxin will include the human-like immunoglobulins of the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

The human-like antibodies and pharmaceutical compositions thereof of this invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously. The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania (1980).

The antibodies of this invention can be lyophilized for storage and reconstituted in suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins and art-known lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution

can lead to varying degrees of antibody activity loss (e.g., with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted to compensate.

The compositions containing the present human-like antibodies or a cocktail thereof can administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the infection and the general state of the patient's own immune system, but generally range from about 1 to about 200 mg of antibody per dose, with dosages of from 5 to 25 mg per patient being more commonly used. It must be kept in mind that the materials of this invention may generally be employed in serious disease states, that is life-threatening or potentially life-threatening situations. In such cases, in view of the minimization of extraneous substances and the lower probability of "foreign substance" rejections which are achieved by the present human-like antibodies of this invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these antibodies.

In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend upon the patient's state of health and general level of immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per patient. A preferred prophylactic use is for the prevention of kidney transplant rejection.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the patient.

Human-like antibodies of the present invention can further find a wide variety of utilities *in vitro*. By way of example, the exemplary antibodies can be utilized for T-cell typing, for isolating specific IL-2 receptor bearing cells or fragments of the receptor, for vaccine preparation, or the like.

For diagnostic purposes, the antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the human-like antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluoros, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of a cellular activity or for the presence of a selected antigen. Thus, the subject antibody composition of the present invention may be provided, usually in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for the desired cell type. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where a second antibody capable of binding to the chimeric antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above.

The following examples are offered by way of illustration, not by limitation.

EXPERIMENTAL

Design of genes for human-like light and heavy chains

The sequence of the human antibody Eu (Sequences of Proteins of Immunological Interest, Kabat, E., et al., U.S. Dept. of Health and Human Services, 1983) was used to provide the framework of the humanized antibody, because the amino acid sequence of the heavy chain of anti-Tac is more homologous to the heavy chain of this antibody than to any other heavy chain sequence in the National Biomedical Foundation Protein Identification Resource.

To select the sequence of the humanized heavy chain, the anti-Tac heavy chain sequence was aligned with the sequence of the Eu heavy chain (Figure 1). At each position, the Eu amino acid was selected for the humanized sequence, unless that position fell in any one of the following categories, in which case the anti-Tac amino acid was selected.

(1) The position fell within a complementarity determining region (CDR), as defined by Kabat, et al., *op. cit.* (amino acids 31-35, 50-66, 99-106);

(2) The Eu amino acid was unusual for human heavy chains at that position, whereas the anti-Tac amino acid was typical for human heavy chains at that position (amino acids 27, 93, 95, 98, 107-109, 111);

(3) The position was immediately adjacent to a CDR in the amino acid sequence of the anti-Tac heavy chain (amino acids 30 and 67).

(4) 3-dimensional modeling of the anti-Tac antibody suggested that the amino acid was physically close to the antigen binding region (amino acids 48 and 68).

Some amino acids fell in more than one of these categories but are only listed in one.

To select the sequence of the humanized light chain, the anti-Tac light chain sequence was aligned with the sequence of the Eu light chain (Figure 2). The Eu amino acid was selected at each position, unless the position again fell into one of the categories (1) - (4), (with light chain replacing heavy chain in the category definitions):

(1) CDRs (amino acids 24-34, 50-56, 89-97).

(2) Anti-Tac amino acid more typical than Eu (amino acids 48 and 63).

(3) Adjacent to CDRs (no amino acids; Eu and anti-Tac were already the same at all these positions).

(4) Possible 3-dimensional proximity to binding region (amino acid 60).

The actual nucleotide sequence of the heavy (Figure 3) and light chain (Figure 4) genes were selected as follows:

(1) the nucleotide sequences code for the amino acid sequences chosen as described above.

(2) 5' of these coding sequences, the nucleotide sequences code for a leader (signal) sequence, namely the leader of the light chain of the antibody MOPC 63 and the leader of the heavy chain of the antibody PCH 108A (Kabat et al., *op. cit.*). These leader sequences were chosen as typical of antibodies.

(3) 3' of the coding sequences, the nucleotide sequences are the sequences that follow the mouse light chain J5 segment and the mouse heavy chain J2 segment, which are part of the anti-Tac sequences. These sequences are included because they contain splice donor signals.

(4) At each end of the sequence is an Xba I site to allow cutting at the Xba I sites and cloning into the Xba I site of a vector.

Construction of humanized light and heavy chain genes

To synthesize the heavy chain, four oligonucleotides HES12, HES13, HES14, HES15 (Figure 5A) were synthesized using an Applied Biosystems 380B DNA synthesizer. Two of the oligonucleotides are part of each strand of the heavy chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 5B). Together, the oligonucleotides cover the entire humanized heavy chain (Figure 3) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

Each oligonucleotide was phosphorylated using ATP and T4 polynucleotide kinase by standard procedures (see, Maniatis, *op. cit.*). To anneal the phosphorylated oligonucleotides, they were suspended together in 40 µl of TA (33 mM Tris acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate) at a concentration of about 3.75 µM each, heated to 95 deg for 4 min. and cooled slowly to 4 °C. To synthesize the complete gene from the oligonucleotides by synthesizing the opposite strand of each oligonucleotide (Figure 5B), the following components were added in a final volume of 100µl:

10 µl	annealed oligonucleotides
0.16 mM each	deoxyribonucleotide
0.5 mM	ATP
0.5 mM	DTT
100 µg/ml	BSA
3.5 µg/ml	T4 g43 protein (DNA polymerase)
25 µg/ml	T4 g44/62 protein (polymerase accessory protein)
25 µg/ml	45 protein (polymerase accessory protein)

The mixture was incubated at 37 °C for 30 min. Then 10 U of T4 DNA ligase was added and incubation at 37 °C resumed for 30 min. The polymerase and ligase were inactivated by incubation of the reaction at 70 °C for 15 min. To digest the gene with Xba I, to the reaction was added 50 µl of 2x TA containing BSA at 200 µg/ml and DTT at 1 mM, 43 µl of water, and 50 U of Xba I in 5 µl. The reaction was incubated for 3 hr at 37 °C, and run on a gel. The 431 bp Xba I fragment was purified from a gel and cloned into the Xba I site of the plasmid pUC19 by standard methods. Four plasmids

isolates were purified and sequenced using the dideoxy method. One of these had the correct sequence (Figure 3).

To synthesize the light chain, four oligonucleotides JFD1, JFD2, JFD3, JFD4 (Figure 6A) were synthesized. Two of the oligonucleotides are part of each strand of the light chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 6B). Together, the oligonucleotides cover the entire humanized light chain (Figure 4) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

The light chain gene was synthesized from these oligonucleotides in two parts. 0.5 µg each of JFD1 and JFD2 were combined in 20 µl sequenase buffer (40 mM Tris-HCl, pH 7.5, 20 mM magnesium chloride, 50 mM sodium chloride), heated at 70 °C for 3 min and allowed to cool slowly to 23 deg in order for the oligonucleotides to anneal. JFD3 and JFD4 were treated in the same way. Each reaction was made 10 mM in DTT and 0.5 mM in each deoxyribonucleotide and 6.5 U of sequenase (US Biochemicals) was added, in a final volume of 24 µl, and incubated for 1 hr at 37 °C to synthesize the opposite strands of the oligonucleotides. Xba I and Hind III were added to each reaction to digest the DNA (there is a Hind III site in the region where JFD2 and JFD3 overlap and therefore in each of the synthesized DNAs; Figure 6B). The reactions were run on polyacrylamide gels, and the Xba I - Hind III fragments were purified and cloned into pUC18 by standard methods. Several plasmid isolates for each fragment were sequenced by the dideoxy method, and correct ones chosen.

Construction of plasmids to express humanized light and heavy chains

The heavy chain Xba I fragment was isolated from the pUC19 plasmid in which it had been inserted and then inserted into the Xba I site of the vector pVγ1 in the correct orientation by standard methods, to produce the plasmid pHuGTAC1 (Figure 7). This plasmid will express high levels of a complete heavy chain when transfected into an appropriate host cell.

The two light chain Xba I - Hind III fragments were isolated from the pUC18 plasmids in which they had been inserted. The vector plasmid pVκ1 was cut with Xba I, dephosphorylated and ligated with the two fragments by standard methods. The desired reaction product has the circular form: vector - Xba I - fragment 1 - Hind III - fragment 2 - Xba I - vector. Several plasmid isolates were analyzed by restriction mapping and sequencing, and one with this form chosen. This plasmid, pHuLTAC (Figure 8), therefore contains the complete humanized light chain (Figure 4) and will express high levels of the light chain when transfected into an appropriate host cell.

Synthesis and affinity of humanized antibody

The plasmids pHuGTAC1 and pHuLTAC were transfected into mouse Sp2/0 cells, and cells that integrated the plasmids were selected on the basis of resistance to mycophenolic acid and/or hygromycin B conferred by the gpt and hyg genes on the plasmids (Figures 7,8) by standard methods. To verify that these cells secreted antibody that binds to the IL-2 receptor, supernatant from the cells was incubated with HUT-102 cells that are known to express the IL-2 receptor. After washing, the cells were incubated with fluorescein-conjugated goat anti-human antibody, washed, and analyzed for fluorescence on a FACSCAN cytofluorometer. The results (Figure 9A), clearly show that the humanized antibody binds to these cells, but not to Jurkat T-cells that do not express the IL-2 receptor (Figure 9D). As controls, the original mouse anti-Tac antibody was also used to stain these cells (Figure 9B,C), giving similar results.

For further experiments, cells producing the humanized antibody were injected into mice, and the resultant ascites collected. Humanized antibody was purified to substantial homogeneity from the ascites by passage through an affinity column of goat anti-human immunoglobulin antibody, prepared on an Affigel-10 support (Bio-Rad Laboratories, Inc., Richmond, CA) according to standard techniques. To determine the affinity of the humanized antibody relative to the original anti-Tac antibody, a competitive binding experiment was performed. About 5×10^5 HUT-102 cells were incubated with known quantities (10 - 40 ng) of the anti-Tac antibody and the humanized anti-Tac antibody for 10 min at 4 °C. Then 100 ng of biotinylated anti-Tac was added to the cells and incubated for 30 min at 4 °C. This quantity of anti-Tac had previously been determined to be sufficient to saturate the binding sites on the cells, but not to be in large excess. Then the cells were washed twice with 2 ml of phosphate buffered saline (PBS) containing 0.1% sodium azide. The cells were then incubated for 30 min at 4 °C with 250 ng of phycoerythrin-conjugated avidin, which bound to the biotinylated anti-Tac already bound to the cells. The cells were washed again as above, fixed in PBS containing 1% paraformaldehyde, and analyzed for fluorescence on a FACSCAN cytofluorometer.

Use of increasing amounts (10 - 40 ng) of the anti-Tac antibody as competitor in the first step decreased the amount of biotinylated anti-Tac that could bind to the cells in the second step, and therefore the amount of phycoerythrin-conjugated avidin that bound in the last step, thus decreasing fluorescence (Figure 10A). Equivalent amounts (20 ng) of anti-Tac, and humanized anti-Tac used as competitor decreased the fluorescence to approximately the same degree (Figure 10B). This shows that these antibodies have approximately the same affinity (within 3 to 4 fold), because if one had much greater affinity, it would have more effectively competed with the biotinylated anti-Tac, thus decreasing fluorescence more.

Biological properties of the humanized antibody

For optimal use in treatment of human disease, the humanized antibody should be able to destroy T-cells in the body that express the IL-2 receptor. One mechanism by which antibodies may destroy target cells is antibody-dependent cell-mediated cytotoxicity, abbreviated ADCC (Fundamental Immunology, Paul, W., Ed., Raven Press, New York (1984), at pg. 681), in which the antibody forms a bridge between the target cell and an effector cell such as a macrophage that can lyse the target. To determine whether the humanized antibody and the original mouse anti-Tac antibody can mediate ADCC, a chromium release assay was performed by standard methods. Specifically, human leukemia HUT-102 cells, which express the IL-2 receptor, were incubated with ^{51}Cr to allow them to absorb this radionuclide. The HUT-102 cells were then incubated with an excess of either anti-Tac or humanized anti-Tac antibody. The HUT-102 cells were next incubated for 4 hrs with either a 30:1 or 100:1 ratio of effector cells, which were normal purified human peripheral blood mononuclear cells that had been activated by incubation for about 20 hrs with human recombinant IL-2. Release of ^{51}Cr , which indicated lysis of the target HUT-102 cells, was measured and the background subtracted (Table 1). The results show that at either ratio of effector cells, anti-Tac did not lyse a significant number of the target cells (less than 5%), while the humanized antibody did (more than 20%). Hence, the humanized antibody is likely to be more efficacious than the original mouse antibody in treating T-cell leukemia or other T-cell mediated diseases.

TABLE 1

Percent ^{51}Cr release after ADCC		
Effector: Target ratio		
	30:1	100:1
Antibody		
Anti-Tac	4%	< 1%
Humanized anti-Tac	24%	23%

From the foregoing, it will be appreciated that the human-like immunoglobulins of the present invention offer numerous advantages of other antibodies. For example, in comparison to anti-Tac mouse monoclonal antibodies, the present human-like IL-2 receptor immunoglobulins can be more economically produced and contain substantially less foreign amino acid sequences. This reduced likelihood of antigenicity after injection into a human patient represents a significant therapeutic improvement for immunoglobulins designed in accordance with the above criteria.

Claims

1. The use of at least one amino acid substitution outside of complementarity determining regions (CDR's) as defined by Kabat et al ("Sequences of Proteins of Immunological Interest", Kabat, E., et al., US Department of Health and Human Services, (1983)) together with Chothia et al (Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)) in the production of a humanized immunoglobulin, wherein said amino acid substitution is from the non-CDR variable region of a non-human donor immunoglobulin, and in which humanized immunoglobulin the variable region amino acid sequence other than the CDR's comprises at least 70 amino acid residues identical to an acceptor human immunoglobulin variable region amino acid sequence, and the CDR's are from the variable region of said non-human donor immunoglobulin.
2. A use according to claim 1, wherein said humanized immunoglobulin is specifically reactive with p55 Tac protein, is capable of inhibiting binding of human interleukin-2 (IL-2) to a human IL-2 receptor, or is capable of binding to a human IL-2 receptor.
3. A use according to claim 2, wherein said humanized immunoglobulin exhibits a binding affinity to a human IL-2 receptor of about 10^8 M^{-1} or stronger.
4. A use according to claim 2 or claim 3, wherein the mature light and heavy variable region protein sequences of said humanized immunoglobulin are homologous to the mature protein sequences in Figures 3 and 4.
5. A use according to any one of claims 1 to 4, wherein said humanized immunoglobulin is an IgG₁ immunoglobulin isotype.

6. A use according to any one of claims 1 to 5, wherein said substitution is immediately adjacent a CDR.
7. A method of producing a humanized immunoglobulin chain having a framework region from a human acceptor immunoglobulin and complementarity determining regions (CDR's) from a donor immunoglobulin capable of binding to an antigen, said method comprising substituting at least one non-CDR framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from the donor immunoglobulin at a position in the immunoglobulin where:
 - (a) the amino acid in the human framework region of the acceptor immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or
 - (b) the amino acid is immediately adjacent to one of the CDR's; or
 - (c) the amino acid is predicted to have a side chain atom capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.
8. A method according to claim 7, wherein there are at least three of said non-CDR framework amino acids substituted by amino acids from the donor immunoglobulin chosen by criteria (a), (b) or (c).
9. A method according to claim 8, wherein at least one of the amino acids substituted from the donor is immediately adjacent to a CDR.
10. A method according to any one of claims 7 to 9, wherein the mature light and heavy variable region protein sequences of said humanized immunoglobulin are homologous to the mature protein sequences in Figures 3 and 4.
11. A humanized immunoglobulin chain obtainable by a use according to any one of claims 1 to 6.
12. A humanized immunoglobulin chain obtainable by a method according to any one of claims 7 to 10.
13. A humanized immunoglobulin in which the heavy and light chains are chains according to claim 11 or claim 12.
14. A polynucleotide comprising a first sequence coding for a human-like immunoglobulin non-CDR framework region and a second sequence coding for one or more CDR's, wherein upon expression said polynucleotide encodes an immunoglobulin chain of claim 11 or claim 12.
15. Polynucleotides according to claim 14 which upon expression encode the chains constituting an immunoglobulin of claim 13.
16. A cell line transfected with a polynucleotide or polynucleotides of claim 14 or claim 15.
17. A process for the preparation of a humanized immunoglobulin as defined in claim 13, which process comprises cultivating a cell line as defined in claim 16 and isolating the humanized immunoglobulin from the cell culture medium.
18. The use of an immunoglobulin of claim 13 or a binding fragment thereof in the manufacture of a medicament.
19. A use according to claim 18 in which the medicament is suitable for treating T-cell mediated disorders in a human patient.
20. A pharmaceutical preparation which contains a humanized immunoglobulin according to claim 13 formulated in a pharmaceutically acceptable form.
21. A pharmaceutical preparation according to claim 20 and for the treatment of T-cell mediated disorders.

Patentansprüche

1. Die Verwendung von mindestens einer Aminosäure-Substitution außerhalb der die Komplementarität bestimmen-

- den Regionen (CDR's), wie bei Kabat et al. ("Sequences of Proteins of Immunological Interest", Kabat, E., et al., US Department of Health and Human Services, (1983)) zusammen mit Chothia et al (Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)) definiert, für die Herstellung von einem humanisierten Immunglobulin, in dem die Aminosäure-Substitution von der nicht-CDR-variablen Region von einem nicht menschlichen Donor-Immunglobulin ist, und in dem die Aminosäuresequenz der variablen Region des humanisierten Immunglobulins anders als die CDR's mindestens 70 Aminosäurereste identisch zu einer Aminosäuresequenz der variablen Region von einem menschlichen Akzeptor-Immunglobulin umfassen, und in dem die CDR's von der variablen Region von dem nicht menschlichen Donor-Immunglobulin sind.
2. Verwendung nach Anspruch 1, wobei das humanisierte Immunglobulin spezifisch reaktiv für p55 TAC-Protein ist, fähig ist, die Bindung von menschlichem Interleukin-2 (IL-2) zu einem menschlichem IL-2-Rezeptor zu verhindern, oder zur Bindung zu einem menschlichen IL-2-Rezeptor fähig ist.
 3. Verwendung nach Anspruch 2, wobei das humanisierte Immunglobulin eine Bindungsaffinität zu einem menschlichen IL-2-Rezeptor von etwa 10^8 M^{-1} oder mehr zeigt.
 4. Verwendung nach Anspruch 2 oder 3, wobei die Proteinsequenzen der leichten und schweren variablen Region von dem humanisierten Immunglobulin homolog zu der Sequenz des reifen Proteins in Figur 3 und 4 sind.
 5. Verwendung nach einem der Ansprüche 1 - 4, wobei das humanisierte Immunglobulin ein IgG₁ Immunglobulin-Iso-
typ ist.
 6. Verwendung nach einem der Ansprüche 1 - 5, wobei die Substitution unmittelbar an eine CDR angrenzt.
 7. Ein Verfahren zur Herstellung einer humanisierten Immunglobulinkette mit einer Rahmenregion von einem menschlichen Akzeptor-Immunglobulin und mit die Komplementarität bestimmenden Regionen (CDR's) von einem Donor-Immunglobulin, die fähig sind, an ein Antigen zu binden, wobei das Verfahren die Substitution von mindestens einer nicht-CDR-Rahmen-Aminosäure des Akzeptor-Immunglobulins durch eine korrespondierende Aminosäure von dem Donor-Immunglobulin an einer Position in den Immunglobulinen umfaßt, wobei:
 - (a) die Aminosäure in der menschlichen Rahmenregion des Akzeptor-Immunglobulins selten für diese Position ist und die korrespondierende Aminosäure in dem Donor-Immunglobulin häufig für diese Position in menschlichen Immunglobulinsequenzen ist, oder
 - (b) die Aminosäure unmittelbar benachbart zu einer der CDR's ist oder
 - (c) die Aminosäure ein Seitenkettenatom hat, das fähig ist, mit dem Antigen oder mit den CDR's des humanisierten Immunglobulins zu interagieren.
 8. Ein Verfahren nach Anspruch 7, in dem mindestens drei der nicht CDR-Rahmen-Aminosäuren substituiert sind durch Aminosäuren des Donor-Immunglobulins, ausgewählt nach den Kriterien (a), (b) oder (c).
 9. Ein Verfahren nach Anspruch 8, wobei mindestens eine der durch den Donor substituierten Aminosäuren unmittelbar an eine CDR angrenzt.
 10. Ein Verfahren nach einem der Ansprüche 7 - 9, wobei die Proteinsequenzen der reifen leichten und schweren variablen Region dieses humanisierten Immunglobulins homolog sind zu den Sequenzen des reifen Proteins in Figur 3 und 4.
 11. Eine humanisierte Immunglobulinkette, erhältlich bei einer Verwendung nach einem der Ansprüche 1 - 6.
 12. Eine humanisierte Immunglobulinkette, erhältlich bei einem Verfahren nach einem der Ansprüche 7 - 10.
 13. Ein humanisiertes Immunglobulin, in dem die schweren und leichten Ketten Ketten nach Anspruch 11 oder 12 sind.
 14. Ein Polynucleotid umfassend eine erste Sequenz kodierend für eine nicht- CDR-Rahmenregion eines menschen-ähnlichen Immunglobulins und eine zweite Sequenz kodierend für eine oder mehrere CDR's, wobei das Polynucleotid eine Immunglobulinkette nach Anspruch 11 oder 12 kodiert.

15. Polynucleotide nach Anspruch 14, die die Ketten kodieren, die ein Immunglobulin nach Anspruch 13 bilden.
16. Eine Zelllinie transferiert mit einem Polynucleotid oder Polynucleotiden nach Anspruch 14 oder Anspruch 15.
- 5 17. Ein Verfahren zur Herstellung von einem humanisierten Immunglobulin nach Anspruch 13, wobei das Verfahren die Kultivierung einer Zelllinie nach Anspruch 16 und die Isolierung des humanisierten Immunglobulins aus dem Zellkulturmedium umfaßt.
- 10 18. Die Verwendung eines Immunglobulins des Anspruchs 13 oder eines Bindungsfragments davon für die Herstellung eines Medikaments.
19. Die Verwendung nach Anspruch 18, wobei das Medikament für die Behandlung von T-Zellen-bedingter Störungen bei einem Menschen geeignet ist.
- 15 20. Eine pharmazeutische Zubereitung, die ein humanisiertes Immunglobulin nach Anspruch 13, zubereitet in einer pharmazeutisch akzeptablen Form, enthält.
21. Eine pharmazeutische Zubereitung nach Anspruch 20 für die Behandlung von T-Zellen-bedingter Störungen.

20

Revendications

- 25 1. Utilisation d'au moins une substitution d'acide aminé hors des régions hypervariables, également dénommées régions "CDR" (Complementary Determining Regions) selon la définition de Kabat et al ("Sequences of proteins of Immunological Interest", Kabat, E., et al., US Department of Health and Human Services, (1983)) ainsi que Chothia et al (Chothia and Lesk, J. MOL. Biol., 196: 901-917 (1987)) pour la production d'une immunoglobuline humanisée, où ladite substitution d'acide aminé provient de la région variable non-CDR d'une immunoglobuline donneur non-humaine et où dans ladite immunoglobuline humanisée, la séquence en acides aminés de la région variable autre que les régions CDR comprend au moins 70 résidus d'acides aminés identiques à la séquence en acides aminés de la région variable d'une immunoglobuline humaine accepteur, et les CDR proviennent de la région variable de ladite immunoglobuline donneur non-humaine.
- 30 2. Utilisation selon la revendication 1 où ladite immunoglobuline humanisée réagit spécifiquement avec la protéine p55 Tac, est capable d'inhiber la liaison de l'interleukine-2 humaine (IL-2) à un récepteur IL-2 humain, ou est capable de se lier à un récepteur humain pour l'IL-2.
- 35 3. Utilisation selon la revendication 2 où ladite immunoglobuline humanisée possède une affinité de liaison pour un récepteur de l'IL-2 humain d'environ 10^8 M⁻¹ ou plus.
- 40 4. Utilisation selon la revendication 2 ou 3 où les séquences protéiques matures de la région variable légère et lourde de ladite immunoglobuline humanisée sont homologues aux séquences protéiques matures des figures 3 et 4.
- 45 5. Utilisation selon l'une quelconque des revendications 1 à 4 où ladite immunoglobuline humanisée est une immunoglobuline d'isotype IgG₁.
- 50 6. Utilisation selon l'une quelconque des revendications 1 à 5 où ladite substitution est immédiatement adjacente à une CDR.
- 55 7. Méthode de production d'une chaîne d'immunoglobuline humanisée ayant une région charpente provenant d'une immunoglobuline accepteur humaine et des régions hypervariables (CDR) provenant d'une immunoglobuline donneur capable de se fixer sur un antigène, ladite méthode comprenant la substitution d'au moins un acide aminé charpente non CDR de l'immunoglobuline accepteur par un acide aminé correspondant provenant de l'immunoglobuline donneur à une position dans les immunoglobulines où:
 - (a) l'acide aminé dans la région charpente humaine de l'immunoglobuline accepteur et rare pour cette position et l'acide aminé correspondant dans l'immunoglobuline donneur et commun pour ladite position dans les séquences d'immunoglobuline humaines; ou
 - (b) l'acide aminé est immédiatement adjacent à l'une des CDR; ou

(c) l'acide aminé est supposé avoir un atome sur une chaîne latérale capable d'interagir avec l'antigène ou avec les CDR de l'immunoglobuline humanisée.

- 5 8. Méthode selon la revendication 7 où il y a au moins trois des dix acides aminés de la région charpente non-CDR qui sont substitués par des acides aminés provenant de l'immunoglobuline donneur choisie en fonction des critères (a), (b) ou (c).
- 10 9. Méthode selon la revendication 8 où au moins l'un des acides aminés substitués provenant du donneur est immédiatement adjacent à un CDR.
- 10 10. Méthode selon l'une quelconque des revendications 7 à 9 où les séquences protéiques matures des régions variables légère et lourde de ladite immunoglobuline humanisée sont homologues aux séquences protéiques matures des figures 3 et 4.
- 15 11. Chaîne d'immunoglobuline humanisée qui peut être obtenue par une utilisation selon l'une quelconque des revendications 1 à 6.
- 20 12. Chaîne d'immunoglobuline humanisée qui peut être obtenue par une méthode selon l'une quelconque des revendications 7 à 10.
- 20 13. Immunoglobuline humanisée dans laquelle les chaînes lourdes et légères sont des chaînes selon les revendications 11 ou 12.
- 25 14. Polynucléotide comprenant une première séquence codant pour une région charpente non-CDR d'une immunoglobuline semblable à une région humaine ("human-like") et une seconde séquence codant pour une ou plusieurs CDR où lors de son expression ledit polynucléotide code une chaîne d'immunoglobuline selon la revendication 11 ou la revendication 12.
- 30 15. Polynucléotides selon la revendication 14 qui lors de leur expression codent les chaînes constituant une immunoglobuline selon la revendication 13.
- 35 16. Lignée cellulaire transfectée avec un polynucléotide ou des polynucléotides selon la revendication 14 ou 15.
- 35 17. Procédé pour la préparation d'une immunoglobuline humanisée tel que défini dans la revendication 13 comprenant la culture d'une lignée cellulaire telle que définie dans la revendication 16 et l'isolement de l'immunoglobuline humanisée du milieu de culture cellulaire.
- 40 18. Utilisation d'une immunoglobuline selon la revendication 13 ou d'un fragment de liaison de celle-ci pour la fabrication d'un médicament.
- 40 19. Utilisation selon la revendication 18 où le médicament est adapté au traitement des désordres médiés par des cellules T chez un patient humain.
- 45 20. Préparation pharmaceutique qui contient une immunoglobuline humanisée selon la revendication 13 formulée sous une forme pharmaceutiquement acceptable.
- 50 21. Préparation pharmaceutique selon la revendication 20 et pour le traitement des désordres médiés par des cellules T.

50

55

1	Q	V	Q	L	Q	Q	S	G	A	E	L	A	K	P	G	A	S	V	K	M
1	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V
21	S	C	K	A	S	G	Y	T	F	T	S	Y	R	M	H	W	V	K	Q	R
21	S	C	K	A	S	G	G	T	F	S	R	S	A	I	I	W	V	R	Q	A
							*			*										
41	P	G	Q	G	L	E	W	I	G	Y	I	N	P	S	T	G	Y	T	E	Y
41	P	G	Q	G	L	E	W	M	G	G	I	V	P	M	F	G	P	P	N	Y
								*												
61	N	Q	K	F	K	D	K	A	T	L	T	A	D	K	S	S	S	T	A	Y
61	A	Q	K	F	Q	G	R	V	T	I	T	A	D	E	S	T	N	T	A	Y
							*	*												
81	M	Q	L	S	S	L	T	F	E	D	S	A	V	Y	Y	C	A	R	G	
81	M	E	L	S	S	L	R	S	E	D	T	A	F	Y	F	C	A	G	G	Y
												*		*			*			
100	G	G	V	F	D	Y	W	G	Q	G	T	T	L	T	V	S	S			
101	G	I	Y	S	P	E	E	Y	N	G	G	L	V	T	V	S	S			
							*	*	*		*									

FIG. 1.

1	Q	I	V	L	T	Q	S	P	A	I	M	S	A	S	P	G	E	K	V	T	
1	D	I	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	T	
21	I	T	C	S	A	S	S	S	I		S	Y	M	H	W	F	Q	Q	K	P	
21	I	T	C	R	A	S	Q	S	I	N	T	W	L	A	W	Y	Q	Q	K	P	
40	G	T	S	P	K	L	W	I	Y	T	T	S	M	L	A	S	G	V	P	A	
41	G	K	A	P	K	L	L	M	Y	K	A	S	S	L	E	S	G	V	P	S	
								*												*	
60	R	F	S	G	S	G	S	G	T	S	Y	S	L	T	I	S	R	M	E	A	
61	R	F	I	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	
			*																		
80	E	D	A	A	T	Y	Y	Y	C	H	Q	R	S	T	Y	P	L	T	F	G	S
81	D	D	F	A	T	Y	Y	C	Q	Q	Y	N	S	D	S	K	M	F	G	Q	
100	G	T	K	L	E	L	K														
101	G	T	K	V	E	V	K														

FIG. 2.

10 20 30 40 50 60
TCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTGCACT
M G W S W I F L F L L S G T A G V H

70 80 90 100 110 120
CTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTGAAGG
S Q V Q L V Q S G A E V K K P G S S V K

130 140 150 160 170 180
TCTCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACAGGATGCACTGGGTAAGGCAGG
V S C K A S G Y T F T S Y R M H W V R Q

190 200 210 220 230 240
CGCCTGGACAGGGTCTGGAATGGATTGGATATATTAATCCGTCCACTGGGTATACTGAAT
A P G Q G L E W I G Y I N P S T G Y T E

250 260 270 280 290 300
ACAATCAGAAGTTCAAGGACAAGGCAACAATTACTGCAGACGAATCCACCAATACAGCCT
Y N Q K F K D K A T I T A D E S T N T A

310 320 330 340 350 360
ACATGGAAGTGAAGCCTGAGATCTGAGGACACCGCAGTCTATTACTGTGCAAGAGGGG
Y M E L S S L R S E D T A V Y Y C A R G

370 380 390 400 410 420
GGGGGGTCTTTGACTACTGGGGCCAAGGAACCCTGGTCACAGTCTCCTCAGGTGAGTCCT
G G V F D Y W G Q G T L V T V S S

430
TAAACCTCTAGA

FIG._3.

10 20 30 40 50 60
 TCTAGATGGAGACCGATACCGTCCTGCTATGGGTCCTGCTATGGGTCCGAGGATCAA
 M E T D T L L L W V L L L W V P G S
 70 80 90 100 110 120
 CCGGAGATATTCAGATGACCCAGTCTCCATCTACCGTCTCTGCTAGCGTCGGGGATAGGG
 T G D I Q M T Q S P S T L S A S V G D R
 130 140 150 160 170 180
 TCACCATAACCTGCTCTGCCAGCTCAAGTATAAGTTACATGCACTGGTACCAGCAGAAGC
 V T I T C S A S S S I S Y M H W Y Q Q K
 190 200 210 220 230 240
 CAGGCAAAGCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTG
 P G K A P K L L I Y T T S N L A S G V P
 250 260 270 280 290 300
 CTGGCTTCAGTGGCAGTGGATCTGGGACCGAGTTCACCGTCACAATCAGCTCTCTGCAGC
 A R F S G S G S G T E F T L T I S S L Q
 310 320 330 340 350 360
 CAGATGATTTGCCCACTTATTACTGCCATCAAAGGAGTACTTACCGACTCAGTTCCGGTC
 P D D F A T Y Y C H Q R S T Y P L T F G
 370 380 390 400
 AGGGGACCAAGGTGGAGGTCAAACGTAAGTACACTTTTCTAGA
 Q G T K V E V K

FIG._4.

A

HES12 AGCTTCAGATGGGATGGAGCTGGATCTTTCTCTTCCCTCCTGTCAAGGTACGGCGGGCGGTG
 CACTCTCAGGTCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAACTGGCTCGAGCGGTG
 AAGGTC

HES13 GCGAGTGGAGGGATTAAATATATCCAAATCCATTCAGAGCCGTGTCCAGGGGGCTTGCCTTAC
 CGAGTGCATCCTGTAGCTAGTAAGGTGTAGCCAGAGCCGTGCGAGGAGACCTTCACGCT
 CGAGCGCAGG

HES14 TATAATTCGTCGAGCTGGGTATACCTGAAATACAGAGTTTCAGGGACAAGGCAACA
 ATTACTGCAGACGGAATCCACCAATACAGCCCTAGATGGAACTGAGCAGCCCTGAGATCTGAG
 GACA

HES15 ATATCGCTCAGAGGTTTTAAGGACTCAGCTGAGGAGACTGTGAGCAGGGTTTCCTTGGCCC
 CAGTAGTCAAGAGACCGCGCGCTGTGACAGTAATAGACTGGGGTGTCCCTCAGATCTC
 AAGGCTGCT

B



FIG. 5.

A

JFD1
CAAACTCTAGATGGAGACGGATACCGCTCGCTGCTATGCGTCCGTCGACGGA
TCAACGGGAGATATTGAGATGACCCAGTCTCCATCTACCCCTCTCTGCTAGCGTCGCGGAT

JFD2
ATAAAATTAGAAAGCTTGGGAGCTTTGGCTGGCTTCTGCTGGTACCAAGTGCAATGTAACCTTAT
ACTTGAAGCTGGCAGAGCAGGTTATGGTGACCCCTATCGCGGACGCTAGCAGAGAG

JFD3
GCTGCCAAGCTTCTAAATTTATACGACATCCAAGCTGGCTTCTGGAGTCCCTGCTCGCTTC
AGTGGCAGTGGATCTGGGACCGAGTTACCCCTCACAATCAGCTCTCTGCGCCGAGATGAT
TTG

JFD4
TATACTAGAAAAGTGTAATTACGTTTGACCTCCACCTTGGTCCCTGACCGAAGCTGAG
TGGGTAAGTACTCCTTTGATGGCAGTAATAAGTGGCGAAATCATCTCTGCTGCGAGAGAGCT
GA

88

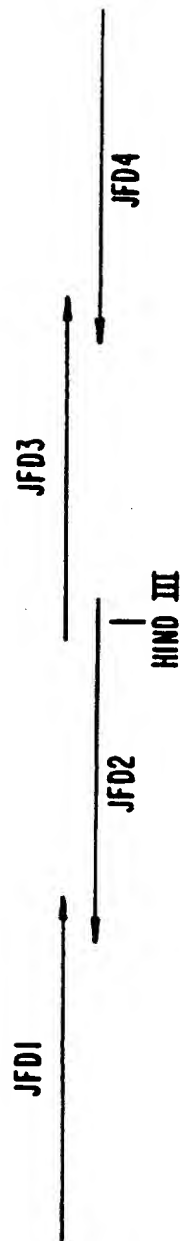


FIG. 6.

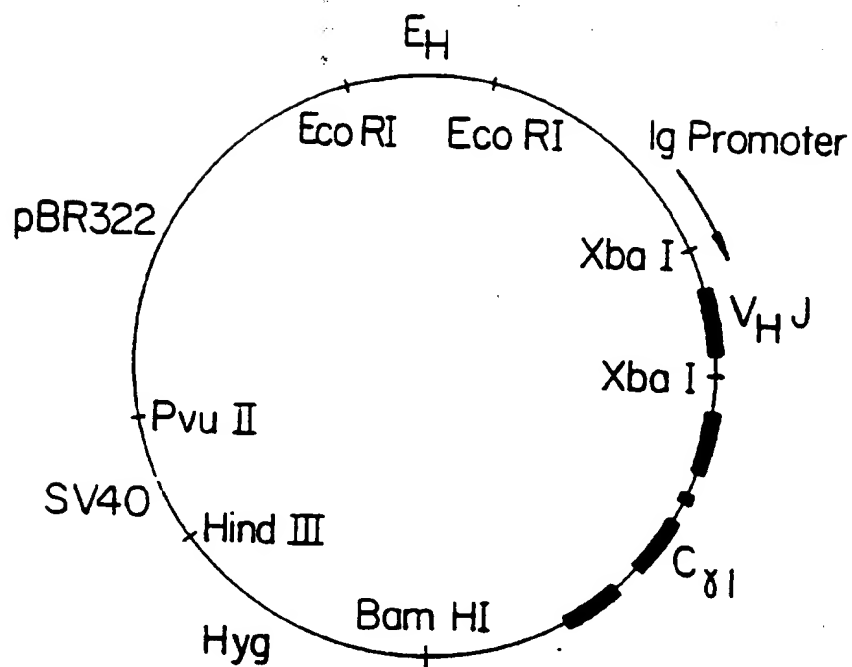


FIG. 7.

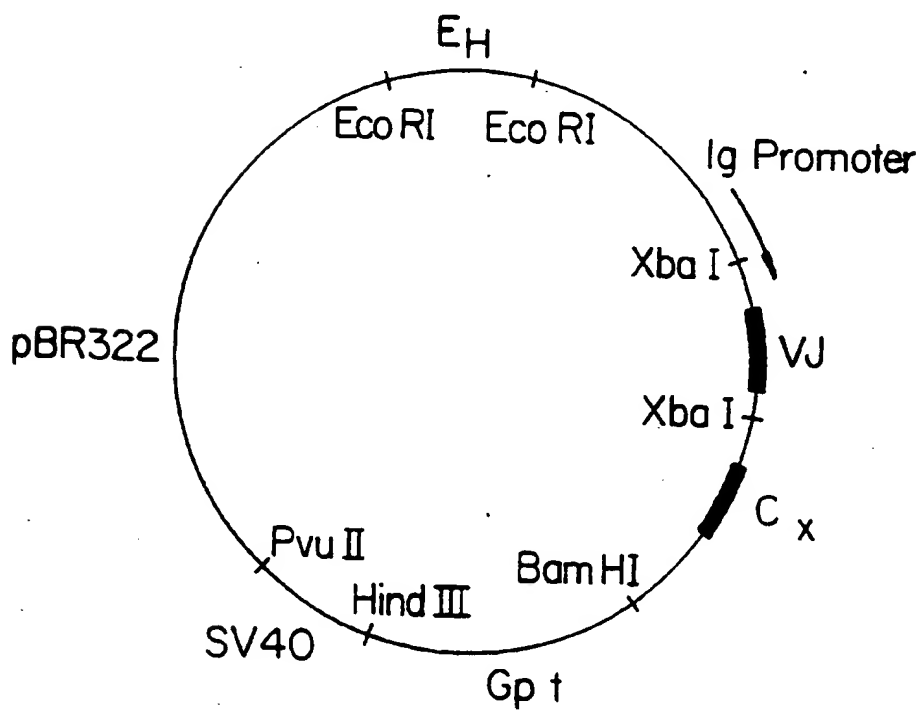


FIG. 8.

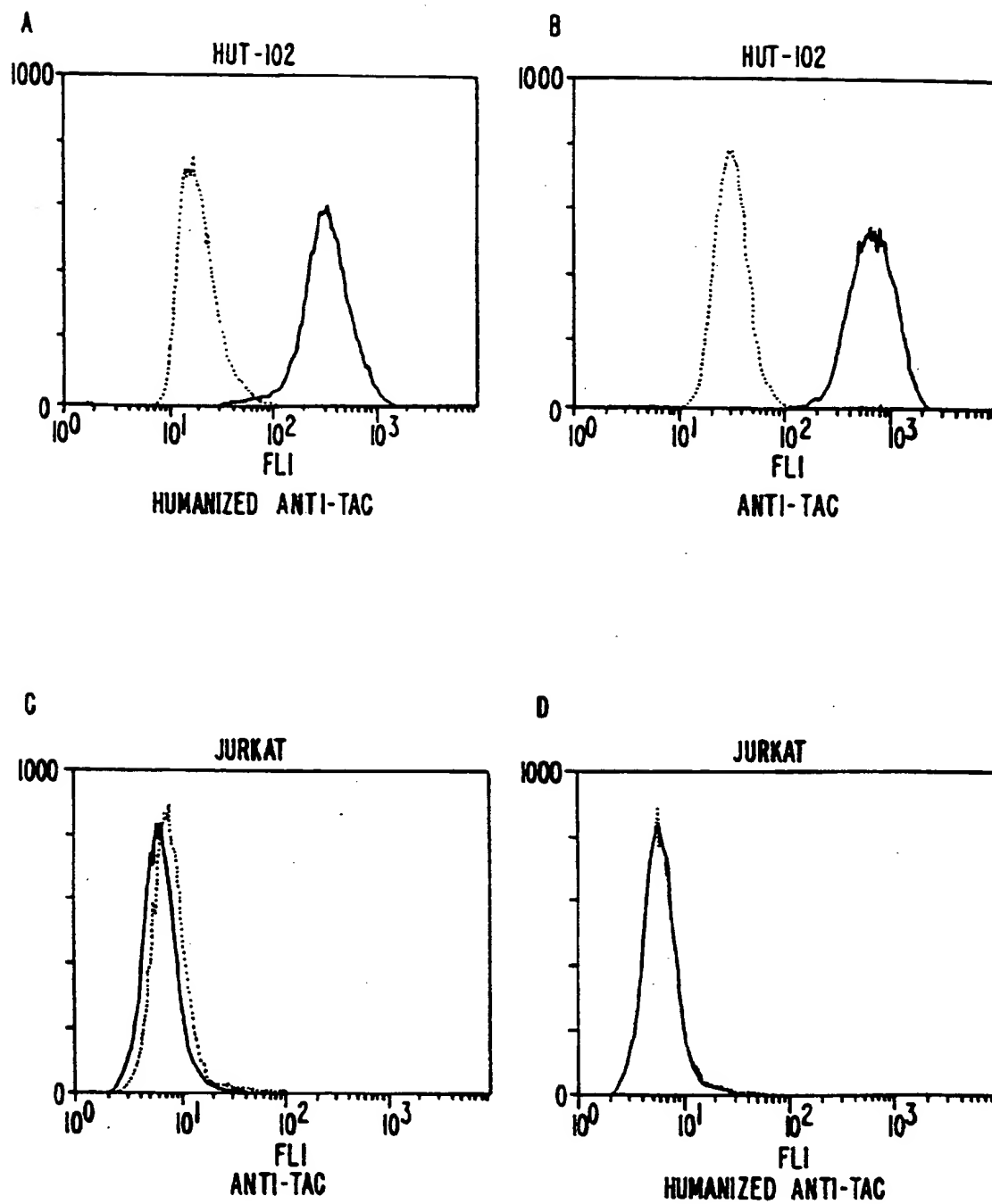


FIG. 9.

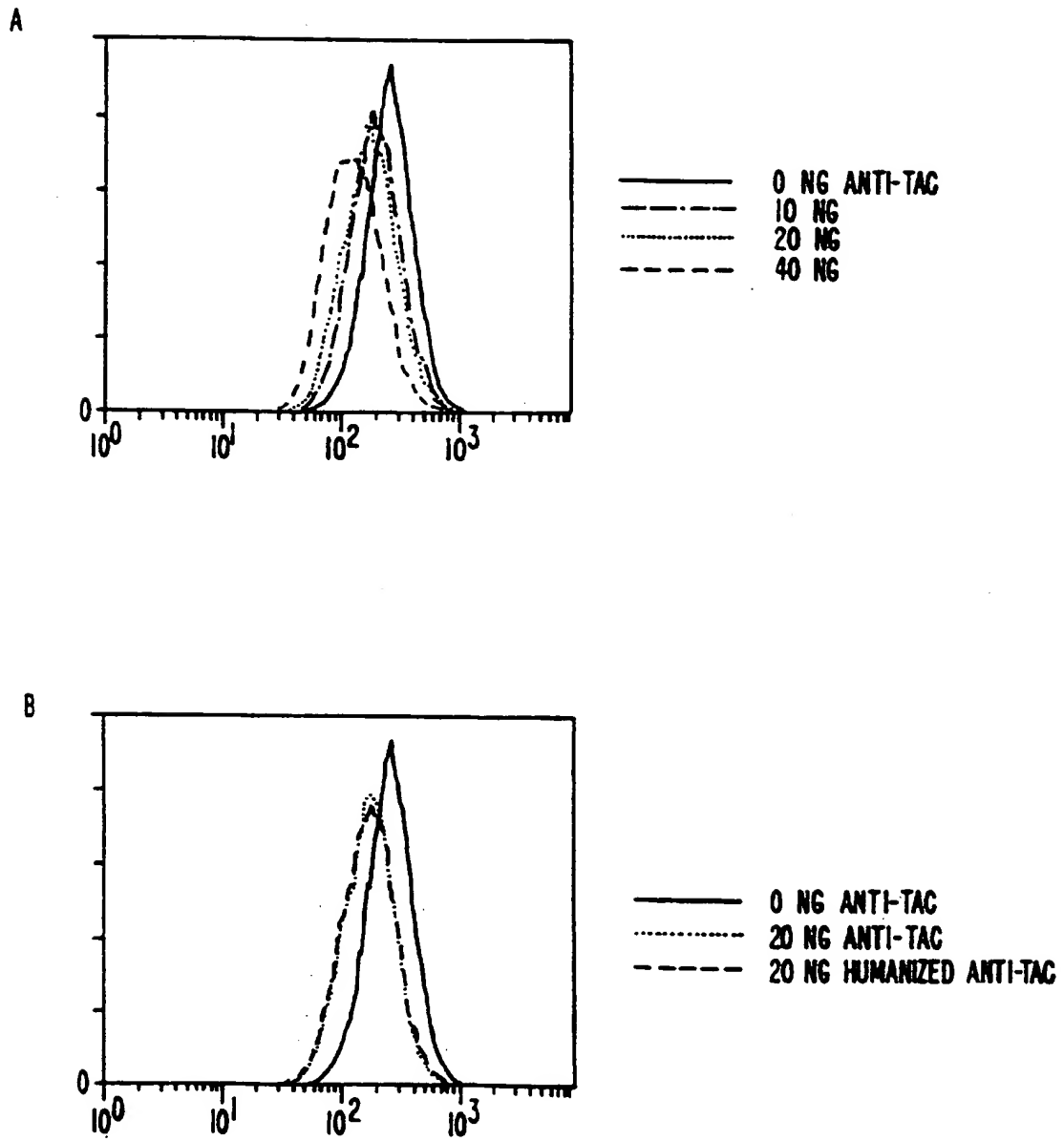


FIG. 10.

DOCKET NO.: CARP-0057

PATENT

RESPONSE UNDER 37 CFR 1.116
EXPEDITED PROCEDURE
EXAMINING GROUP NO. 1642

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658**

Group No.: **1642**

Filed: **May 1, 1997**

Examiner: **M. Davis**

For: **HUMANISED ANTIBODIES**

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to Box AF, Assistant Commissioner for Patents, Washington, D.C. 20231.

On May 20, 2002
Doreen Yatko Trujillo
Doreen Yatko Trujillo, Reg. No. 35,719

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

REQUEST FOR RECONSIDERATION

Pursuant to 37 C.F.R. § 1.116, Applicants request reconsideration and withdrawal of the sole remaining rejection in this application. A Final Rejection was issued December 18, 2001. A petition for a two-month extension of time, and the appropriate fee, accompany this request.

Claims 24-31 are pending. Claims 24-31 were again rejected as allegedly unpatentable under 35 U.S.C. § 102(e) in view of U.S. Pat. No. 5,585,089 (the "Queen patent"). Applicants respectfully traverse the rejection.

Preliminarily, Applicants thank the Examiner and her Supervisor for the very helpful interview conducted on January 31, 2002. During that interview, the rejection of claims 24-31 under 35 U.S.C. § 102(e) in view of the Queen patent was discussed. As Applicants stated during the interview, the Queen patent is not entitled to its earliest priority dates. Thus, it is not an appropriate reference under 35 U.S.C. § 102(e).

To recapitulate, the Queen patent claims priority to four earlier applications, two of which are continuations-in-part. For the Queen patent to be entitled to the earliest priority dates as a reference under 35 U.S.C. § 102(e), there must be support for the claims *as allowed* in those priority applications, i.e., the claims must comply with 35 U.S.C. § 112 as of those earlier filing dates, including the written description requirement. (See MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981).) Applicants have maintained that several of the limitations recited in the claims as allowed in the Queen patent do not find written descriptive support in at least the two earliest Queen priority applications – i.e., Queen priority Application Serial No. 07/290,975, filed **December 28, 1988** ("Queen '975") and Queen priority Application Serial No. 08/310,252, filed **February 13, 1989** ("Queen '252"). If the claim limitations do not find written descriptive support in those earlier applications, then the Queen patent is not an effective reference under 35 U.S.C. § 102(e) because Applicants are entitled to their GB priority date of **December 21, 1989**, which is earlier.

The limitation specifically focused upon during the aforementioned interview was the recitation "outside the Kabat and Chothia CDRs." This limitation is particularly significant because it was required for the Queen patent claims to be allowed. Indeed, it was argued that the claims distinguish over the prior art because the immunoglobulins contain donor amino acids "outside the Kabat and Chothia CDRs." (See Amendment dated May 31, 1996, page 5, of the application which issued as the Queen patent.) Neither the limitation, nor support therefor, however, was present in the claims or applications as originally filed in Queen '975 nor in Queen '252. Indeed, the claims as originally filed referred merely to "CDRs." It is Applicants' position that the patentees did not show possession of the later-claimed invention as of the filing dates of Queen '975 and Queen '252. The Queen patent, thus, is not entitled to those earlier filing dates

under 35 U.S.C. § 102(e).

In the Final Rejection, the Examiner again relied upon a single passage in Queen '975 (Queen '975 was incorporated by reference in Queen '252) to support the position that one of ordinary skill in the art would have recognized that CDRs as taught by Queen would also include CDRs as defined by Chothia et al, "regardless of whether the rest of the specification discloses as examples Kabat's CDR's." (See Final Rejection dated December 18, 2001, page 4.) As stressed during the interview, however, the Examiner's position is contrary to what was argued by the patentees themselves during prosecution of the European equivalents of the Queen patent when faced with rejections/objections similar to the written description requirement of 35 U.S.C. § 112, first paragraph. Notably, the European equivalents claim priority to Queen '975 and Queen '252.

In Queen's European patent 0 451 216 B1 ("the European patent," Exhibit 1), granted claim 1 recited that there was to be "at least one amino acid substitution outside of" CDRs "as defined by Kabat et al . . . together with Chothia et al" The European patent was revoked in its entirety under Article 123(2) of the European Patent Convention, which is duplicated below:

A European patent application or a European patent may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed.

The European Board of Opposition ("European Board") concluded that

the feature *Kabat [...]together with Chothia [...]* in claim 1 has neither a technically reasonable nor a legal basis in the application documents as filed; claim 1 does not therefore meet the requirements of Art. 123(2) EPC.

(See Interlocutory decision in Opposition Proceedings, page 27, Exhibit 4.).¹ The European Board

¹ Like the written description requirement of 35 U.S.C. § 112, first paragraph, implicit support for amendments is also recognized under Art. 123(2) EPC. (See Decision T 0292/85, page 5, and Decision T 1212/97, page 6, Exhibits 2 and 3, respectively.) Clearly, then, the European Board did not find even implicit support for the feature.

interpreted the reference simply to "CDRs" in granted claim 7 without further definition to mean Kabat and Chothia CDRs and, thus, revoked it as well.

In an appeal of the decision revoking the patent, the patentees submitted claims similar to granted claim 7, i.e., referencing simply "CDRs." They argued that, contrary to the finding of the European Board,

. . . unless specifically defining CDR's otherwise as done in granted claim 1, the person skilled in the art when reading the application as filed and the patent specification would have inevitably understood that the CDRs in granted claim 7 referred to Kabat CDRs.

(See paper filed June 22, 2001 by Protein Design Labs in appeal of EP 0 451 216 B1, page 8, Exhibit 5.) The Examiner's position, thus, is inapposite to the patentee's.

Indeed, in a paper filed in the opposition of a divisional patent stemming from the application that issued as the European patent, the patentees further argued that

. . . the skilled person who turned to the experimental example in the contested Patent for guidance in carrying out the method of claim 1 would necessarily interpret CDRs according to the definition of Kabat.

(See paper filed July 13, 2001 by Protein Design Labs in EP 0 682 040 B1, page 7, Exhibit 6.) The patentees went so far as to state that

. . . **nowhere** does the contested Patent state that the Chothia definition is to be used in **carrying** out the invention or in **understanding** the claims.

(*Id.*, page 6, emphasis added.) Claim 1 in the divisional patent recites, simply, "CDRs" (European patent 0 682 040 B1, Exhibit 7).

In view of the foregoing, Applicants respectfully submit that the Examiner's position regarding how one skilled in the art would interpret CDRs in Queen '975 and Queen '252 is not only inconsistent with the specification and contrary to the findings of the European Board,

but it is also contrary to what the patentees themselves argued to overcome revocation in Europe. Patentees should not be afforded one interpretation of the same claim limitation to avoid the prior art in the U.S. (i.e., CDRs mean Kabat plus Chothia), and another interpretation of the same claim limitation to meet, effectively, the written description requirement in Europe (i.e., CDRs mean Kabat only). The analysis in both forums on this point, i.e., written description, is essentially the same. Accordingly, Applicants respectfully request that the rejection of claims 24-31 be withdrawn and an interference between the present application and the Queen patent be declared. The Examiner is requested to contact the undersigned at (215) 564-8352 if she feels a telephonic discussion will be helpful.

Respectfully submitted,



Doreen Yatko Trujillo
Registration No. 35,719

Date: May 20, 2002

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STATEMENT DUE 14.6.01

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Application No. / Patent No. 90 903 576.8-2116 / 0451216 / 04	Ref. 000945EP/CPM/EK	Date 14.02.2001
Proprietor PROTEIN DESIGN LABS, INC.		

Interlocutory decision in Opposition proceedings (Articles 102(3) and 106(3) EPC)

The Opposition Division - at the oral proceedings dated 20.03.2000 - has decided:

Account being taken of the amendments made by the patent proprietor during the opposition proceedings, the patent and the invention to which it relates are found to meet the requirements of the Convention.

The reasons for the decision are enclosed.

Documents for the maintenance of the patent as amended:

Text for the Contracting States:
AT BE CH LI DE ES FR GB IT LU NL SE

Description, pages:

1,2,4,6,8-13	of the patent specification		
3,5,7	as received on	14.06.2000	with letter of 12.06.2000

Claims, No.:

1-13	as received on	17.12.1999	with letter of 17.12.1999
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Drawings, No.:

1-10	of the patent specification
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Comments:

In the letter dated 17.12.99, this auxiliary request was originally designated "5th

Registered letter with advice of delivery



Date 14.02.2001

Sheet 2

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auxiliary request", but has been renumbered in the letter dated 16/03/00 to become "3rd auxiliary request"

Possibility of appeal

This decision is open to appeal. Attention is drawn to the attached text of Articles 106 to 108 EPC.

Opposition Division:

Chairman: WEAVER M R
2nd Examiner: HOESEL H R
1st Examiner: GOETZ M E



Ormerod, A

Formalities Officer

Tel. No.: +49 89 2399-8164

Enclosure(s): 53 page(s) reasons for the decision (Form 2916)
Wording of Articles 106 - 108 (Form 2019)
Documents relating to the amended text
☐ Minutes of oral proceedings
Annex A comprising the complete list of documents submitted
during the Proceedings

to EPO postal service: 07.02.2001

Registered letter with advice of delivery

EPO Form 2327 11.99CSX




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Anmelde-Nr.: Application No.: Demande n°:	90 903 576.8		

I. Summary of Facts and submissions

A Submissions made by the parties during the written phase of the opposition procedure

1. European patent EP-B-0 451 216, based on previous international application WO90/07861 (corresponding European application number 90903576.8), and claiming priority of 28/12/88 (US290 975, "PDL1 application") and 13/02/89 (US310 252, "PDL2 application"), was granted on 24/01/96 to **Protein Design Labs, Inc.** (the Proprietor) with the following claims *inter alia*:

Claim 1:

"The use of at least one amino acid substitution outside of complementarity determining regions (CDR's) as defined by Kabat et al ("*Sequences of Proteins of Immunological Interest*", Kabat, E., et al., US Department of Health and Human Services, (1983)) together with Chothia et al (Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)) in the production of a humanized immunoglobulin, wherein said amino acid substitution is from the non-CDR variable region of a non-human donor immunoglobulin, and in which humanized immunoglobulin the variable region amino acid sequence other than the CDR's comprises at least 70 amino acid residues identical to an acceptor human immunoglobulin variable region amino acid sequence, and the CDR's are from the variable region of said non-human donor immunoglobulin."

Claim 7:

"A method of producing a humanized immunoglobulin chain having a framework region from a human acceptor immunoglobulin and complementarity determining regions (CDR's) from a donor immunoglobulin capable of binding to an antigen, said method comprising substituting at least one non-CDR framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from the donor immunoglobulin at a position in the immunoglobulins where:

- (a) the amino acid in the human framework region of the acceptor immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or
- (b) the amino acid is immediately adjacent to one of the CDR's; or
- (c) the amino acid is predicted to have a side chain atom capable of interacting with the antigen or

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Date 14.02.2001**Grounds for the decision (Annex)**Blatt
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Feuille**Motifs de la décision (Annexe)**Anmelde-Nr.:
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with the CDR's of the humanized immunoglobulin."

Claim 11:

"A humanized immunoglobulin chain obtainable by a use according to any one of claims 1 to 6."

Claim 12:

"A humanized immunoglobulin chain obtainable by a method according to any one of claims 7 to 10."

2. Notices of opposition were filed by the following Opponents:

Name	Designation	Opposition with letter dated
Medical Research Council	Opponent 1	23/09/96
Icos Corporation	Opponent 2	01/10/96
Novartis AG	Opponent 3	08/10/96
Celltech Therapeutics Ltd	Opponent 4	24/10/96
Bayer AG	Opponent 5	23/10/96
Chiron Corporation	Opponent 6	21/10/96
SmithKline Beecham	Opponent 7	23/10/96
Genentech Inc.	Opponent 8	23/10/96
IDEC Pharmaceuticals Corp.	Opponent 9	24/10/96
Biotest Pharma GmbH	Opponent 10	22/10/96
Biotransplant Inc.	Opponent 11	24/10/96
Bristol-Myers Comp.	Opponent 12	24/10/96
Glaxo group Ltd.	Opponent 13	23/10/96
Boehringer Ingelheim GmbH	Opponent 14	24/10/96
Merck Patent GmbH	Opponent 15	21/10/96
Chugai Seiyaku Kabushiki Kaisha	Opponent 16	23/10/96
Schering Corp.	Opponent 17	22/10/96
Ixsys Inc.	Opponent 18	24/10/96

The Opponents requested the revocation either of the patent in toto or only of some of the claims as granted and based their requests on the grounds for opposition as set forth below:

**Addition of subject-matter going beyond the application as originally filed
(Art. 100(c), 123(2) and (3) EPC)**

Opponent 1	objection raised against	claims 1, 7
Opponent 2	----	claims 1, 7 and claims dependent thereupon
Opponent 4	----	claims 1, 5, 6 and claims dependent thereupon
Opponent 5	----	claims 1, 5 - 21



Entscheidungsgründe (Anlage)

Grounds for the decision (Annex)

Motifs de la décision (Annexe)

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Opponent 7	--- " ---	claims 1, 11, 12, 17
Opponent 8	--- " ---	claims 1 - 21
Opponent 9	--- " ---	claims 1 - 6, 11, 13 - 21
Opponent 10	--- " ---	claims 1 - 21
Opponent 11	--- " ---	claim 1
Opponent 12	--- " ---	claims 1 - 21
Opponent 13	--- " ---	claims 1 - 21
Opponent 15	--- " ---	claims 1 - 6, 11, 13 - 21
Opponent 16	--- " ---	claim 1
Opponent 17	--- " ---	claim 1

No patentable invention has been made (Art. 100(a) and 52 EPC)

Opponent 7	objection raised against	claims 1, 11
------------	--------------------------	--------------

Lack of novelty (Art. 100(a) and 54 EPC)

Opponent 1	objection raised against	claims 1, 5, 6 - 21
Opponent 2	--- " ---	claims 1, 5 - 7, 9, 11, 13, 14 - 18, 20
Opponent 3	--- " ---	claims 1, 5 - 9, 11 - 17
Opponent 4	--- " ---	claims 1, 5 - 21
Opponent 5	--- " ---	claims 1, 5 - 7, 11 - 18
Opponent 6	--- " ---	claims 1, 7
Opponent 7	--- " ---	claims 1 - 21 when PRIO is invalid
	--- " ---	claims 1, 7, 11 - 13 when PRIO is invalid
Opponent 8	--- " ---	claims 1, 6 - 9, 11 - 19, 21
Opponent 9	--- " ---	claims 1 - 21 when PRIO is invalid
Opponent 10	--- " ---	claims 1 - 21
Opponent 11	--- " ---	claims 1, 7
Opponent 12	--- " ---	claims 1, 6 - 9, 11 - 21
Opponent 13	--- " ---	claims 1, 5, 11, 13 - 18
Opponent 14	--- " ---	claims 1, 5, 7, 8, 10 - 17
Opponent 15	--- " ---	claims 1, 6 - 9, 11 - 21
Opponent 16	--- " ---	claims 1, 7
Opponent 18	--- " ---	claims 1, 7

Lack of inventive step (Art. 100(a) and 56 EPC)

Opponent 1	objection raised against	claims 1, 5, 6, 7, 9, 11 - 21
Opponent 2	--- " ---	claims 1 - 21
Opponent 3	--- " ---	claims 1, 5 - 9, 11 - 21
Opponent 4	--- " ---	claims 1 - 21
Opponent 5	--- " ---	claims 1 - 21
Opponent 6	--- " ---	claims 1 - 21
Opponent 8	--- " ---	claims 1, 3 (when dependent on claim 1), 5, 20
Opponent 9	--- " ---	claims 1 - 21
Opponent 10	--- " ---	claims 1 - 21
Opponent 11	--- " ---	claims 1 - 21
Opponent 12	--- " ---	claims 1, 7
Opponent 13	--- " ---	claims 1 - 21
Opponent 14	--- " ---	claims 1 - 21
Opponent 15	--- " ---	claims 1 - 21
Opponent 16	--- " ---	claims 1 - 21
Opponent 17	--- " ---	claims 1 - 21

**Entscheidungsgründe (Anlage)****Grounds for the decision (Annex)****Motifs de la décision (Annexe)**

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Opponent 18 ---- " ---- claims 1 - 7, 10 - 21

Lack of entitlement to priority

Opponent 1	objection raised against	claims 1, 7
Opponent 3	---- " ----	claims 1, 7 and claims dependent thereupon
Opponent 4	---- " ----	claims 1, 5 - 21
Opponent 5	---- " ----	claims 1, 7 and claims dependent thereupon
Opponent 7	---- " ----	claims 1, 11
Opponent 8	---- " ----	claims 1, 5 - 9, 11 - 21
Opponent 9	---- " ----	claims 1 - 21
Opponent 10	---- " ----	claims 1 - 21
Opponent 11	---- " ----	claim 1
Opponent 15	---- " ----	claims 1 - 21 (first claimed priority date)

Insufficient disclosure (Art. 100(b) and 83 EPC)

Opponent 2	objection raised against	claims 1 - 21
Opponent 3	---- " ----	claims 1, 7 and claims dependent thereupon
Opponent 4	---- " ----	claims 1, 7, 19
Opponent 5	---- " ----	claims 1, 7, 19
Opponent 7	---- " ----	claims 1, 11
Opponent 8	---- " ----	claims 19, 21
Opponent 10	---- " ----	claims 1 - 21
Opponent 12	---- " ----	claim 1
Opponent 13	---- " ----	claims 1 - 21
Opponent 16	---- " ----	claims 1, 7
Opponent 18	---- " ----	claims 1 - 21

In the event that the Opposition Division should not be in a position to comply with their requests, all 18 Opponents requested that Oral proceedings be scheduled.

Documents **D1 - D87** listed in Annex A to this decision were submitted by the Opponents in their respective statement of opposition.

3. With a letter dated 07/04/98, the Proprietor submitted a first reply to the above oppositions wherein he refutes the arguments brought forward by the Opponents and requests maintenance of the patent as granted. Oral Proceedings were requested in the event that the Opposition Division should not be in a position to comply with his request.

Documents **D88 - D102** listed in Annex A were submitted by the Proprietor in support of his position.



4. Observations were made by the third party "Buzz Lightyear" on 12/05/98 and 07/12/99. Following the EPO's practice as set out in the Formalities Officer's letter to the Proprietor dated 18/06/98, these observations were considered to be acceptable.
5. On 12/05/99, the invitation to attend Oral Proceedings was sent out, together with an annex setting out the preliminary opinion of the Opposition Division on the issues raised by the Opponents and the Proprietor, as summarized below:
- On the ground "Added subject-matter": claims 1, 2 - 6 and 11 were considered not meet the requirements according to Art. 123(2) EPC. Deletion of the unallowably added subject-matter was considered to result in an unallowable broadening of their scope (Art. 123(3) EPC).
 - On the issue "Lack of entitlement to priority": for claims 1 - 6 and 11 no opinion was expressed because of their non-compliance with Art. 123(2)/(3). For claim 7, only the 2nd priority date of 13/02/89 was considered to be validly claimed.
 - On the ground "Lack of novelty": for claims 1 - 6 and 11 no opinion was expressed because of their non-compliance with Art. 123(2)/(3). Claims 7, 9, 12 - 17 having an effective date of 13/02/89 (second priority of PDL2 application) were not considered to meet the requirements according to Art. 54(1) - (4) EPC in view of D36 and D48.
 - On the ground "Lack of inventive step": the Opposition Division considered it to be obvious to take into consideration any suggestion relating to a possible role of Kabat framework amino acid residues in the antigen binding (D32, D23, D38, D40) in the light of the teaching provided by D36.
 - On the ground "Insufficient disclosure": The granted patent was considered to be in accordance with the requirements pursuant to Art. 83 EPC.
 - On the ground "Non-patentable subject-matter": The granted patent was acknowledged to be in accordance with the requirements pursuant to Art. 52(2)(a) - (c) EPC.

Additionally, the decisions of the EPO's Technical Boards of Appeal D103 - D111 as set out in Annex A were identified.



Entscheidungsgründe (Anlage)

Grounds for the decision (Annex)

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6. Observations on the preliminary opinion issued by the Opposition Division were submitted by the Proprietor in a letter dated 17/12/99, together with a set of 5 auxiliary requests. In support of his position, the Proprietor further submitted the documents **D112 - D129** as listed in Annex A.
7. Further substantial observations, partly on the preliminary opinion issued by the Opposition Division and partly on the letter of the Proprietor dated 17/12/99, were submitted by:
 - Opponent 4 on 16/12/99, the submission introducing further documents **D130 - D134** as listed in Annex A.
 - Opponent 8 on 17/12/99, 08/03/00 and 13/03/00, the submission introducing further documents **D135 - D141** as listed in Annex A.
 - Opponent 16 on 22/01/99
 - Opponent 17 on 11/02/00
8. With a letter dated 16/03/00, the Proprietor submitted a new set of 3 auxiliary requests, wherein auxiliary requests 1 and 2 were derived from auxiliary requests 3 and 4 as filed on 17/12/99 and auxiliary request 3 was identical with auxiliary request 5 as filed on 17/12/99. Briefly, the amendments made in the set of auxiliary requests are:

First auxiliary request

- Claim 1 is based on claim 7 as granted, with the following modifications:
 - "Framework" replaced by "**Kabat framework**"
 - The element "**... and wherein at least one of said amino acid substitution is also outside of the first heavy chain hypervariable loop as defined by Chothia et al. [Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)] ...**" has been added.
- Claims 2 - 14 are adapted correspondingly, with minor modifications in their wording.

**Second auxiliary request**

- Claim 1 is as in the first auxiliary request, but the amino acid selection criterion is a restricted version of criterion (c) only ("... amino acids having a side chain **capable of interacting with the CDR ...**" instead of "**capable of interacting with the CDR or with the antigen ...**")
- Claims 2 and 3 correspond to claim 1 of the first auxiliary request, but the selection criterion for amino acids are criteria (a) and (b) only.
- Claims 4 - 16 are adapted correspondingly, with minor modifications in their wording.

Third auxiliary request

- Claim 1 corresponds to claim 7 as granted, but includes the subject-matter of claim 10 as granted, reciting the specific amino acid sequence of the anti-Tac antibody.
 - Claims 2 - 13 were adapted correspondingly, with minor modifications in their wording.
9. With letters of 21/12/99, 02/03/00, 17/03/00 and with telefax of 20/03/00, Opponents 1, 7, 16 and 15 withdrew their oppositions; with telefaxes of 08/03/00, 09/03/00, 15/03/00, 10/03/00, and 18/02/00, Opponents 2, 9, 12, 13 and 18 declared that they did not wish to attend the Oral Proceedings.
10. Oral Proceedings were held on 20/03/00. The main request as well as auxiliary requests 1 and 2 were rejected for non-compliance with Art. 123(2) EPC; the patent was maintained on the basis of auxiliary request 3 which was found to comply with the requirements of the EPC.

B Submissions made by the parties during the Oral Proceedings

After rejection of the main request as well as auxiliary requests 1 and 2 for non-compliance with Art. 123(2) EPC, the Proprietor requested the Opposition Division to set forth the exact reasons for the rejection; he furthermore requested an opportunity to file another auxiliary request which would take into account the said reasons. In reply, some of the Opponents announced that, should the Opposition Division be inclined to accept the Proprietor's requests, they would seek for an adjournment of the Oral Proceedings and an award of costs.

For the reasons set out below, the Opposition Division refused both requests of the Proprietor.



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II. Reasons for the decision

A The main request - Art. 123(2) and (3) EPC

During prosecution of the case, the following objections were raised by the Opponents:

1. Although claim 1 as granted has the sole technical requirement that *"... at least 70 amino acid residues ..."* of the human acceptor framework be present in **the whole Ig molecule**, such element did not allegedly exist in the application as filed, where it was clearly stated that at least 70 human amino acid should be present **per Ig chain** in a humanized immunoglobulin. As it is known that a complete Ig molecule has four chains, the said requirement in claim 1 would appear to constitute added subject-matter.

The Opposition Division cannot share this view: the relevant passage in the description of the application as filed reads (page 11/lines 3 - 7)

"As used herein, a "human-like framework region" is a framework region that in each existing chain comprises at least about 70 or more amino acid residues, typically 75 to 85 or more residues, identical to those in a human immunoglobulin."

However, claim 1 also states that in the humanized immunoglobulin which it is desired to protect

*"... the **variable region** amino acid sequence other than the CDR's comprises at least 70 amino acid residues identical to an acceptor human immunoglobulin **variable region** amino acid sequence,";*

and therefore clearly indicates that the occurrence of 70 amino acid residues is not a technical feature of the whole immunoglobulin molecule, but that it relates to each variable region amino acid sequence, **such variable region occurring in each of the 4 chains constituting an immunoglobulin molecule.**



This would appear to correlate perfectly with the Proprietor's definition of an immunoglobulin, which includes single heavy or light chains, see page 10/lines 10 - 24 of the application as filed.

Hence, it is considered that the "... *at least 70 amino acid residues* ..." feature of claim 1 as granted does not constitute an addition of previously undisclosed subject-matter.

2. Whereas claim 22 as originally filed related to "*A Humanized immunoglobulin designed according to claims 18, 19 or 20*", the Opponents noted that the corresponding claims 11 and 12 of the patent used the term "*obtainable by*" instead of "*designed*". This would allow for the claimed humanized immunoglobulin to be produced by other methods than the methods of claims 1 - 10 (to which claims 11 and 12 refer), said methods clearly not having been disclosed in the application as filed.

However, as correctly pointed out by the Proprietor, it is not appropriate to stipulate that the scope of claims to be granted should not be broader than the scope of the claims as originally filed; indeed, Art. 123(2) EPC only prescribes that an application may not be amended in such a way that it contains subject-matter extending beyond the content of the application (claims, description, drawings) as filed.

Claims 11 and 12 find ample support in the description as originally filed, see e.g. page 4/lines 1 - 36 (note the use of the terms "*may*" and "*can*" in the characterization of the immunoglobulins of the application), page 6/lines 21 - 26 and page 9/lines 2 - 5. Moreover, the structure of the immunoglobulins according to the alleged invention is defined in method claims 1 - 6 and 7 - 10; claims 11 and 12 are product claims which merely refer to the immunoglobulins defined in the method claims, independently of the production method.

Hence, the specification as originally filed (description, claims and drawings) is considered by the Opposition Division to provide sufficient substantial support for the subject-matter of the claims 11 and 12, bearing in mind the common knowledge level of the skilled practitioner.



3. Although claim 1 as granted recites "... *at least one amino acid substitution ...*" outside of CDRs without any further limitation, the Opponents have argued that the same technical feature has always been linked in the application as originally filed to certain criteria according to which an amino acid residue must be selected for substitution. Thus, claim 19 as originally filed would clearly teach that at least one human framework amino acid can be substituted **when and only when** it meets one of criteria (a) to (c) as further set forth in claim 19. This would appear to be confirmed by the passage bridging pages 5 and 6 of the application as filed.

Moreover, it would appear that the wording "... *at least one amino acid substitution ...*", as used in granted claim 1 and on page/lines 14 - 20, unallowably adds subject-matter because the application as filed does not disclose the production of a humanized immunoglobulin with less than 3 amino acid substitutions, such as e.g. an Ig molecule where only 1 amino acid residue has been substituted.

The Opposition Division cannot share this view: it should be pointed out that the technical element of granted claim 1, "... *at least one amino acid substitution outside of CDRs ... in the production of a humanized immunoglobulin, ...*" is supported by claim 11 (page 35/lines 14 - 19) and claim 19 as originally filed (page 36/lines 27 and 28),

*"... said method comprising the steps of substituting **at least one** amino acid of the acceptor immunoglobulin ...",*

and the statement made on page 5, lines 32 - 36 of the description as filed.

Contrary to the view expressed by some of the Opponents, the statement of page 5, lines 32 - 36 of the description as filed would have been interpreted by the skilled person as a "stand-alone" description of a preferred embodiment relating to the substitution of amino acids as such, without an immediate link to technical selection criteria. Indeed, the next sentence starts with "*More specifically, ...*", indicating that the following statement relates to the further, restricted embodiment of selecting the amino acid residues to be substituted by following criteria (a) - (c) as set out on page 6/lines 3 - 13.



Hence, the specification as originally filed is considered by the Opposition Division to provide sufficient substantial support for the "... *at least one amino acid substitution* ..." feature of claim 1 as granted.

4. Although claim 7 as granted recites substitution criterion (c) as comprising the sole technical requirement that the amino acid should have a side chain atom capable of interacting with the antigen or with the CDRs of the humanized immunoglobulin, the Opponents stated that, in the application as filed, the same criterion (c) was mandatorily linked to the technical element of the same amino acid being also "... *within about 3 Å of the CDRs* ...", as seen from page 6 / lines 10 - 13, page 14 / lines 14 - 25 and claim 19. Omission of the latter feature in claim 7 as granted was therefore seen as a violation of Art. 123(2) EPC.

As demonstrated by the Proprietor in the letter dated 07/04/98, page 13, said objection is considered to be without merit.

Indeed, in the Opposition Division's view, the omission of the technical element "... *within about 3 Å of the CDRs* ..." from original claim 19, now claim 7, does not contravene Art. 123(2) EPC, as the description as filed, in the explanation of "Criterion IV" on page 14, already clearly pointed out that the said element is merely a secondary feature of the main criterion telling the reader that certain amino acids have been shown to have the capability of interacting with the antigen or the CDR via certain mechanisms.

Indeed, page 14, lines 21 - 25 of the description as filed read

*"Amino acids according to this criterion will **generally** have a side chain within about 3 Ångstrom units ... and must contain atoms that could interact ..."*,

(emphasis added), thereby indicating that the second criterion, "... *side chain containing atoms that could interact* ..." is obligatory, whereas the first criterion, "... *within about 3 Å* ..." does not necessarily apply, a view which appears to be supported by the experimental details given for the humanized anti-*Tac* antibody, see page 26/lines 35 - 38 and page 27/lines 15 - 16 of the description as filed.



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5. Although claim 1 as granted recites that "... said amino acid substitution is from the non-CDR variable region of a non-human donor immunoglobulin, ...", some Opponents alleged that the original application did not provide such basis for claiming any possible donor sequence with the exception of human sequences. Indeed, it would appear that the application as filed disclosed only murine donor sequences, as e.g. seen from page 4/lines 16 - 20 and page 5/lines 34 - 36, such murine sequences not constituting an adequate support for an extension of this technical element to "non-human" (i.e. comprising any other animal with the exception of humans) sequences.

This objection must already fail in view of the technical principle underlying the claimed subject-matter, as set out throughout the whole application as filed. It is clear to any skilled person that humanized antibodies can only be prepared by grafting foreign (i.e., non-human) stretches of amino acids onto a human acceptor framework, as explained on e.g. page 5/lines 8 - 12 of the application as filed:

"The present invention also provides novel methods for designing human-like immunoglobulin chains having one or more complementarity determining regions (CDR's) from a donor immunoglobulin and a framework region from a human immunoglobulin ..."

This passage clearly does not restrict the donor sequences to amino acid stretches selected from murine antibodies, which is only a preferred embodiment of the claimed subject-matter, as can be seen from e.g. page 4 lines 20 - 23, reading

"For example, mouse complementarity determining regions, with or without additional naturally-associated mouse amino acid residues, can be used ..."

Hence, it is considered that the use of a sequence selected from a "... non-human donor immunoglobulin ..." is disclosed in the application as originally filed.



6. All Opponents have vigorously attacked the patent as granted under Art. 123(2) EPC on the ground that claim 1 comprises the Proprietor's own definition of a complementarity determining region (CDR),

"... complementarity determining regions (CDR's) as defined by Kabat et al ("Sequences of Proteins of Immunological Interest", Kabat, E., et al., US Department of Health and Human Services, (1983)) together with Chothia et al (Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)) in the production of a humanized immunoglobulin, ...".

More particularly, the Opponents objected to the use of the term *"together with"* linking the two references to prior art documents containing the two alleged definitions of CDRs, as the application as filed did not provide any basis for such additive re-definition, which conferred CDR1 (the complementarity determining region of an antibody's heavy chain variable region) the meaning of a stretch of amino acids extending from residue 26 to residue 35. Indeed, as explained in the Proprietor's submissions, the aim of such proprietary definition of CDRs was clearly to distinguish claim 1 (and claim 7) from document **D36**, wherein *Riechmann et al.* disclose a reshaped antibody comprising CDRs of rat antibody YTH 34.5HL on a human framework, and where intentional Ser27 ---> Phe27 and Ser30 ---> Thr30 framework substitutions were made.

a) Preliminary remark

It is quite clear that the incorporation of Applicant's proprietary CDR definition into claim 1 as granted, *"CDRs as defined by Kabat (...) together with Chothia (...)"*, can by no means be discussed under the mere aspect of "simple addition of a reference to the prior art" as alleged by the Proprietor. Since, depending on its interpretation, it is suitable to substantially modify the scope of the claims (amino acid residues 26 - 30 included in CDR1 of the heavy chain or not) **and** the relevance of previously published documents such as **D36**, the said definition must be considered to be a relevant technical feature of the claimed subject-matter.

In this context, attention is drawn to the fact that the incorporation of the feature *"Chothia definition of CDRs"*, as allegedly disclosed in prior art



document **D28**, into claim 1 (in an additive manner to the scientifically accepted definition provided by *Kabat*, see paragraphs d1) - d6) below) does not comply with the rules set out for such incorporation in EPC Guidelines C-VI, 5.7d. Indeed, at least rule (i) given in the Guidelines is not complied with, as it was not undoubtedly clear that, from the simple citation of the *Chothia* document on page 10/lines 5 - 6 of the application as filed, protection was possibly sought for any feature comprised in the said document.

- b) As argued by the Proprietor, the alleged basis for his own definition of a CDR is the unique part of the application as filed which relates to CDR definitions and which is on page 9/line 37 - page 10/line 9:

"The variable regions of each light/heavy chain pair form the antibody binding site. The chains all exhibit the same general structure of relatively conserved framework regions joined by three hypervariable regions, also called CDRs (see, "Sequences of Proteins of Immunological Interest," Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987), which are incorporated herein by reference)".

The two references mentioned here, *Kabat et al.* and *Chothia et al.*, correspond to documents **D15** and **D28**, respectively.

On the basis of this originally disclosed passage, the text portions on page 3 of the patent comprising lines 7 - 25 and lines 29 - 36 have been included in the description; furthermore, a new claim 1 has been submitted for substantive examination, ultimately resulting in claim 1 as granted, comprising the Applicant's own definition of CDR1 of the heavy chain as extending from residues 26 - 35.

- c) The arguments set forth by the Opponents clearly followed two lines:

Objection 1: Neither the application documents as filed (explicitly) nor the state of the art available at the priority date of the subject-matter of claim 1 (implicitly, by way of the reference to documents **D15** and **D28**) provided a basis for a combined



"Kabat and Chothia" definition of CDRs, because *Chothia* **had not attempted to define CDRs**. In such case, violation of Art. 123(2) EPC would have to be seen in the addition of a new technical teaching for which no scientific support whatsoever would exist.

Objection 2: Assuming, *arguendo*, that a technical basis for a combined "Kabat and Chothia" CDR definition were to exist, the wording of the sole passage quoting **D15** and **D28**, together with the remainder of references to "CDR" or "Framework" amino acid residues in the application as filed, would not allow for an **additive combination** of a CDR as defined by Kabat in D15 (extending from residues 31 - 35 of the V_H chain) with a CDR as defined by Chothia in D28 (extending from residues 26 - 32 of the V_H chain) into a "new" CDR as defined by the Proprietor (extending from residues 26 - 35 of the V_H chain).

d) Concerning objection 1:

It has to be decided whether *Chothia et al.* have made an attempt to provide **an alternative or refined definition** of the CDR definition given by *Kabat et al.* and, if so, whether the skilled person would have acknowledged that such redefinition had been made.

Having analysed the considerable amount of arguments and evidence, mainly in the form of sworn declarations made by eminent scientists, which has been submitted by the Proprietor and the Opponents to support their case, the Opposition Division has come to the conclusions as set forth below.

d1) A redefinition of Kabat CDRs by *Chothia* ?

Document **D97**, submitted by the Proprietor, provides what appears to be a clue to a correct interpretation:



"The 27-30th and 94th residues of V_H framework's of mouse B-B10 were also grafted, since these residues were considered to affect the conformation of the CDR's (Chothia and Lesk, 1987 (D28); Chothia et al., 1989 (D29))."

D97, page 438

"The 27-30th and 94th amino acid residues of humanized B-B10 V_H were grafted from the corresponding amino acid residues of mouse B-B10, despite the fact that these residues belonged to framework's. The framework residues to be reverted were chosen according to Chothia and Lesk, 1987 (D28); Chothia et al., 1989 (D29) who pointed out that the particular framework residues participated in or had an influence on the formation of correct CDR loops."

D97, page 442

The underlined terms in these three citations, i.e. "*the conformation of the CDR's*" and "*CDR loops*" support the Opposition Division's view that CDRs as defined by *Kabat* represent **numbered linear sequences of amino acids** forming the hypervariable regions of an antibody. Parts of the said hypervariable regions may assume a particular three-dimensional conformation and form the "**hypervariable loops**" according to *Chothia*. Hence, basically, as noted in **D97**, the primary CDR structures (*Kabat*) assume a secondary loop conformation (*Chothia*).

The only common element to the *Kabat* and the *Chothia* approach would appear to reside in their attempt to provide a characterization of **hypervariable regions**.

d2) The view adopted by *Kabat et al.*

In their basic document **D7**, they introduce the technical term "*CDR*" to describe the hypervariable regions of an antibody as the amino acid stretches "*making contact with the antigen*"; CDRs are defined in terms of the **variability in their primary amino acid sequence**, based on previous work done by *Wu & Kabat* in **D1**, where the said variability is mathematically defined.



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D7 depicts the 6 CDRs of the light and heavy chain variable region, using the nomenclature

CDR1 - 3 of V_L: amino acid residues 24 - 34, 50 - 56 and 89 - 97
CDR1 - 3 of V_H: amino acid residues 31 - 35, 50 - 65 and 95 - 102

The designation "*Framework residues*" is used for all other amino acid residues.

Taking a somewhat extreme position, *Kabat et al.* have not done anything more than to identify and number the hypervariable amino acid residues at the primary structure level of the immunoglobulin. D15, D27 and D62 repeat this definition (see e.g. D15, pages ii, iii, vi and x) and give a tabular overview of published CDRs.

d3) The view adopted by *Chothia et al.*

In D28, *Chothia* seeks a characterisation of the hypervariable regions in terms of their three-dimensional structure; to this end, they analyse the amino acid responsible for the **conformation** of hypervariable loops connecting the β -sheet framework of the variable region.

The authors themselves point out that their approach to predicting the three-dimensional structure of the hypervariable loops is different from previous work of scientists having made antibody modelling studies (to which group of scientists *Kabat* also belongs): instead of using the CDR approach (based on linear **sequence variability**), they define the amino acid residues which are responsible for the main chain **conformation**: they find that most of the hypervariable loops have one of a small discrete set of possible main chain conformations ("*canonical structures*"). It turns out that beside residues in the *Kabat* CDRs, also other residues in the *Kabat* framework have influence on the **conformation** of the loops. Hence, five of *Chothia*'s canonical structures are **shorter** and therefore represent a sequence subset of the *Kabat* CDRs (CDR1 - 3 of the light chain, CDR2 and 3 of the heavy chain), one is **overlapping** with *Kabat* CDR1 of the heavy chain.



As a logical consequence of this different approach, *Chothia* never uses the term "CDR" to designate hypervariable loops: instead the nomenclature L1 - L3 (light chain hypervariable loops) and H1 - H3 (heavy chain hypervariable loops) is used, see also the *Chothia* declaration **D84**.

Most importantly, *Chothia* himself has made the strict distinction between *Kabat* CDR's and his hypervariable loops:

"Their limits are somewhat different from those of the CDR's defined by Kabat et al. (1983) on the basis of sequence variability: ..."

D28, page 904,

and in **D24**,

"The antigen-binding site contains three hypervariable loops from V_L and three from V_H , denoted L1, L2, L3 and H1, H2, H3 (11). The residues, numbered according to Kabat et al. (5), are: ... [The complementarity determining regions (CDRs), defined by Kabat et al. (5) on the basis of sequence comparisons, are more extensive]."

D24, page 755

d4) The view adopted by the scientific community

As pointed out by the Proprietor, it is to be recognized that much of the scientific literature published has added a lot of confusion by having interchangeably used two designations for what should have been considered to represent technically different features, i.e. "CDR1 - 3 of V_H and V_L " as opposed to "Hypervariable loops H1 - H3 and L1 - L3".

Compare for instance, a citation made in **D40**:

*"... in CDR1 of the antibody heavy chain which extends from residues 31 to 35 by sequence (Kabat, **D27**) and from residue 26 - 32 in structural terms (Chothia, **D28**) ..."*

D40, page 172



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with the statement given in **D36**:

*"By sequence, the first hypervariable loop extends from residues 31 - 35 (ref. 25, **D27**), whereas by structure it extends from residues 26 - 32 (ref. 32, **D28**)."*

***D36**, page 326*

Under the aspect of technical nomenclature, both statements (and any other such statement found in the literature) appear to be inaccurate, as *Kabat* has not defined "loops", but linear amino acid sequences and *Chothia* has never defined CDRs, but hypervariable loops.

It appears that any correct reference to a delineation of hypervariable regions must take into account the existence of two technically distinct approaches:

- "CDR" must be equated to the *Kabat* definition of hypervariable region based on sequence variability and hence always means the **numbered** sequences consisting of residues 24 - 34, 50 - 56 and 89 - 97 (light chain) and residues 31 - 35, 50 - 65 and 95 - 102 (heavy chain);
- "Hypervariable loop" must relate to *Chothia*'s three-dimensional structural considerations of the hypervariable region, including the observation that residues outside of the *Kabat* framework have influence on the **conformation** of the loops.

Such technically accurate references are numerous, see the same document **D40** as above, where it is confirmed that a Ser27 ---> Phe27 mutation made by *Riechmann* in **D36** occurred

"... in the antibody 'framework' region, in addition to the CDR replacement steps, ..."

***D40**, last 3 lines of page 172, left column,*

see furthermore **D26**, describing that

"... The individual β -strands are linked by loops which at one tip of the β -sheet may fashion a binding pocket for small haptens^{1,2}. Sequence comparisons among heavy- and light-chain variable domains (...) reveal that each domain has three CDR's flanked by



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four relatively conserved regions (framework regions)⁴. As seen in the structure of the human myeloma protein NEWM (Fig.1), the CDRs include each of the three main loops. ..."

D26, page 522,

from which statement it is apparent that the author makes a clear distinction between "CDRs" and "loops",

see **D32**, reading

"The determination as to what constitutes a CDR and what constitutes a framework region was made on the basis of the amino acid sequences of a number of Igs. However, from the 3-dimensional structure of a number of Igs, it is apparent that the antigen binding site of an Ig variable domain comprises three looped regions supported on sheet-like structures. The loop regions do not correspond exactly to the CDR's, although in general there is considerable overlap."

D32, page 6, last paragraph,

see **D48**, noting that

"Although the two changes Ser(27) to Phe and Ser(30) to Thr are located within the framework region as defined in reference 11 (D27), they lie within the hypervariable loop H1 as defined in reference 18 (D28):"

D48, page 3, lines 40 - 42,

and see also **D69**, stating *expressis verbis* that

"The framework residues 28 - 30 are part of the H1 hypervariable loop (residues 26 to 32) defined by Chothia & Lesk."

D69, page 57, bottom of left column,

In the Opposition Division's view, and although some scientists such as the Declarants nominated by the Proprietor may have a different view, the overwhelming majority of publications referring to CDRs correctly use this term as defined by *Kabat*, i.e. they consider it as a numbering system of linear sequences consisting of the hypervariable amino acid residues 24 - 34, 50 - 56 and 89 - 97 (CDRs of the light chain) and residues 31 - 35, 50 - 65 and 95 - 102 of the heavy chain (CDRs of the heavy chain),



see **D10**, page 684/Table 1, **D23**, page 750/Tables 1 and 3, **D32**, page 17/4th paragraph and Figs. 2, 3 and 7, **D36**, page 325/right column/lines 4 - 5 of the section "Strategy" and Fig. 1, **D38**, Fig. 3, **D48**, Figs. 2a and 2b, see also the documents submitted by the Proprietor, **D91**, Fig. 6, **D94**, Figs. 1a, 1b, 2a, 2b and 12, **D96**, Fig. 3.

It is worthy of note that publications having the inventors as co-authors also clearly show that they understand the term "CDR" as defined by *Kabat*, see **D52**, page 10031/right column, Fig. 2 and **D56**, Fig. 1 and Table 1.

Last, but not least, the present inventors also had no difficulties in accepting the *Kabat* CDR definition in the relevant passages of the disputed patent, as will be demonstrated in section d6) below.

d5) Conclusion

There is no convincing evidence for an alternative or refined definition of *Kabat et al.* CDRs by *Chothia et al.*: the latter authors simply establish the concept of the "hypervariable loop" by determining the **residues controlling the 3-dimensional conformation** as opposed to *Kabat's* definition of a CDR strictly relying on **residues determining the sequence variability**. To this end, *Chothia* accepted the CDR definition of *Kabat* based on sequence hypervariability and merely **compared** his data to the *Kabat* CDR's by, of course, using the *Kabat* numbering system.

What **both** authors have done, is a characterization of hypervariable regions: "CDR" is one technical aspect of hypervariable regions, "hypervariable loop" is the other one.

It follows that, according to the skilled person's understanding of the published prior art at the effective date of the application, the statement

"The variable regions of each light/heavy chain pair form the antibody binding site. The chains all exhibit the same general structure of relatively conserved framework regions joined by three hypervariable regions, also called CDRs (see, "Sequences of Proteins of



Immunological Interest," Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987), which are incorporated herein by reference)".

made on page 9 / line 37 - page 10 / line 7 of the application as filed, can by no means be considered to provide a basis for the Proprietor's proprietary definition of CDRs, i.e. equivalent to an additive combination of "CDR" as defined by *Kabat* in **D7** and "loop" according to the *Chothia* document **D28**.

In the light of the relevant prior art as analysed above, the meaning the skilled practitioner would give to this sentence in the original application is clearly that *Kabat* had defined the CDRs on the basis of sequence variability, that *Chothia* had acknowledged this definition and had given a new characterization of **hypervariable regions** by establishing the concept of **hypervariable loops**.

Hence, the reference in claim 1 and the description to *Chothia* (**D28**) as a document defining CDRs is improper and reflects the state of the art in an inaccurate manner; it therefore renders claim 1 unclear (Art. 84 EPC).

d6) The meaning given to "CDR" in the application:

In striking contrast to the signification the Proprietor wishes to attribute to the term "CDR" in claim 1, the remainder of the application as filed is in perfect agreement with *Kabat's* CDR definition:

- The text portion on page 10/line 37 - page 11/line 3 of the application as filed explains that

"As used herein, the term "framework region" refers to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved (i.e., other than the CDR's) among different immunoglobulins in a single species, as defined by Kabat, et al., op. cit.",



and therefore defines the *Kabat* framework residues (**with no reference to *Chothia***) in the commonly accepted technical sense; since page 11/lines 19 - 22 further asserts that

"..., also included are criteria by which a limited number of amino acids in the framework of a humanlike or humanized immunoglobulin chain are chosen to be the same as the amino acids at those positions in the donor Ig rather than in the acceptor Ig, in order to increase the affinity of an antibody comprising the humanized immunoglobulin chain."

this can only mean that amino acid residues in the framework as defined by *Kabat* are to be substituted. However, if the framework is defined according to *Kabat*, the Applicant has implicitly acknowledged the *Kabat* definition of CDRs.

- In the experimental part of the application as filed, the list of 4 selection criteria according to which a human acceptor amino acid at a particular position should be replaced by a non-human donor amino acid comprises a clear acceptance of the *Kabat* CDR definition: at least the light chain CDRs and the crucial CDR1 of the heavy chain are acknowledged to consist of amino acid residues 24 - 34, 50 - 56, 89 - 97 and 31 - 35, as defined by *Kabat* (with no reference to *Chothia*), see e.g. page 26/lines 23 - 26,

"At each position, the Eu amino acid was selected for the humanized sequence, unless that position fell in any one of the following categories, in which case the anti-Tac amino acid was selected:

- (1) The position fell within a complementarity determining region (CDR), as defined by Kabat, et al., op. cit. (amino acids 31-35, 50-66, 99-106);"*

and Figs. 1 and 2, wherein the *Kabat* CDRs are underlined.

- From the indication given on page 26/lines 32 - 34 of the application, that amino acid residue 30 of the heavy chain is "*adjacent to a CDR*", it can only be concluded that CDR1 starts at position 31 and therefore complies with the *Kabat* definition (with no reference to *Chothia*).

e) Concerning objection 2:



Basically following the reasoning set forth by the Third Party and by some Opponents, it may be assumed, *arguendo*, that *Kabat* has defined CDR1 of the heavy chain as being constituted by amino acid residues 31 - 35 and *Chothia* has defined **the same CDR1** as extending from amino acid 26 to amino acid 32.

Clearly, in such a case, the text portion of the description

"The variable regions of each light/heavy chain pair form the antibody binding site. The chains all exhibit the same general structure of relatively conserved framework regions joined by three hypervariable regions, also called CDRs (see, "Sequences of Proteins of Immunological Interest," Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987), which are incorporated herein by reference)".

would then be considered by the skilled person to reflect such an assumption, namely that there existed **two different interpretations of the same entity**, one of them (by *Kabat*) postulating

"CDR1 comprises amino acid residues 31 - 35"

and the other one (by *Chothia*) stating that

"CDR1 extends from amino acid residues 26 - 32"

It goes without saying that the mere provision of a reference to such divergent interpretations (as done in the application by the citation of the two relevant pieces of prior art describing the said interpretations, *Kabat* 1983 and *Chothia* 1987) **does not** however constitute a supporting disclosure of the combination, that is

"In this patent , CDR1 comprises amino acid residues 26 - 32 PLUS residues 31 - 35"

ultimately resulting in the proprietary definition of CDR1 of the heavy chain to comprise amino acid residues 26 - 35. The Proprietor's assertion that such



definition should be accepted because of the fact that the references to the *Kabat* and *Chothia* documents on page 10/lines 4 - 6 of the application as filed are linked by "and" instead of "or", is considered to be without merit, as this is merely a linguistic consideration which ignores the fact that in the English language, the listing of several equivalent, optionally applicable alternatives by using the conjunction "and", does not imply that this listing automatically provides the additive information contained in all items of the list.

7. Conclusion as to Art. 123(2) EPC

From the above analysis, the Opposition Division concludes that the feature "*Kabat [...] together with Chothia [...]*" in claim 1 has neither a technically reasonable nor a legal basis in the application documents as filed; claim 1 does not therefore meet the requirements of Art. 123(2) EPC.


This opinion also applies to claims 2 - 6 directly dependent upon claim 1 and to claim 11 reciting humanized immunoglobulins obtainable by the method of claim 1, as well as to any further claim being dependent upon or referring thereto.

This opinion also applies to claim 7 and 12 where no particular definition of the CDR has been given; however, in the absence of such definition, and since it is assumed that the same invention is under consideration, the Proprietor is clearly bound by the definition he has provided in claim 1.

8. Conclusion as to Art. 123(3) EPC

The unallowably extended subject-matter of claim 1 (and claims 2 - 7, 11, 12, as well as any further claim being dependent upon or referring thereto) of the patent excludes a stretch of the human acceptor antibody comprising amino acid residues 26 - 35 from substitution by a murine amino acid; deletion of the extended subject-matter, thereby reverting to the commonly accepted *Kabat* CDR1 definition, would result in a claim only excluding amino acid residues 31 - 35, equivalent to an unallowable broadening of its scope (Art. 123(3) EPC).

B Auxiliary requests 1 and 2 - Art. 123(2) EPC

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1. Claim 1 of auxiliary requests 1 and 2 are each based on claim 7 as granted, with the following modifications:

- "Framework" has been replaced by "**Kabat framework**"
- The additional element "... and wherein at least one of said amino acid substitution is also outside of the first heavy chain hypervariable loop as defined by Chothia et al. [Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)] ..." has been added (this element will subsequently be abbreviated to read "*at least one AA outside Chothia*").
- Claim 1 of the second auxiliary request differs from claim 1 of the first auxiliary request in that only selection criterion (c) for the amino acids to be substituted is recited.


Claims 2 - 14 in the first auxiliary request and claims 2 - 16 in the second auxiliary request were adapted correspondingly, with minor modifications in their wording.

In the procedure, it became clear that some Opponents objected to the fact that the Proprietor has omitted to clarify whether the "*at least one AA outside Chothia*" feature should be considered to represent a limiting technical feature or a disclaimer. The following analysis takes into account both alternatives.

2. The "*at least one AA outside Chothia*" element as a limiting technical feature:

A limiting technical feature must clearly have a basis in the application as originally filed, in order to comply with Art. 123(2) EPC.

Taking into account the reasons set forth in the rejection of the main request under Art. 123(2) EPC, the Opposition Division is still of the opinion that no such basis exists.

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Indeed, it is still considered that the only part of the application as filed which mentions the *Chothia* article (page 9/line 37 - page 10/line 9), and reading

"The variable regions of each light/heavy chain pair form the antibody binding site. The chains all exhibit the same general structure of relatively conserved framework regions joined by three hypervariable regions, also called CDRs (see, "Sequences of Proteins of Immunological Interest," Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987), which are incorporated herein by reference)".

is nothing more than an acknowledgement of prior art, as required by Rule 27(1)(b) EPC.

Even if it is assumed, *arguendo*, that by such acknowledgement, the teaching given by *Chothia* (i.e. that "his" hypervariable loop H1 extends from amino acid residues 26 - 32, contrasting with *Kabat's* definition of CDR1 as spanning residues 31 - 35) is incorporated into the application as filed, the latter does not contain any support whatsoever of the generalized teaching that any humanized immunoglobulin prepared according to the methods of the application regularly must have *"at least one amino acid substitution ... outside of the first heavy chain hypervariable loop as defined by Chothia et al. [...]"*.

In fact, with regard to the observations already made in paragraph d6) above, the meaning attributed to the term "CDR" throughout the application as filed clearly follows *Kabat's* definition. For instance, the skilled person, looking at Fig. 1 depicting the heavy chain of the single exemplified antibody prepared according to the method of the patent, would not recognize anything more than the substitution of 2 amino acid residues at positions 27 and 30, **outside of the underlined *Kabat* CDR1**. From this unique example, either considered on its own or in conjunction with the body of the description, he/she could not derive the slightest indication that an alleged novel and inventive teaching resides in the regular substitution of an amino acid outside *Chothia's* hypervariable loop H1 extending from amino acid residues 26 - 32.

Hence, the addition of the *"at least one AA outside Chothia"* feature, when seen as a limiting feature, does not comply with the requirements of Art. 123(2) EPC.



3. The "at least one AA outside *Chothia*" feature as a disclaimer:

a) As seen by the Proprietor:

According to the submissions made by the Proprietor, the introduction of the said feature in claim 1 of the first and second auxiliary request meets the basic requirements for acceptability of a disclaimer:

- Firstly, the disclaimer format was considered to be appropriate, as no "positive limitation" could be used to express the alleged novel and inventive teaching that an amino acid outside of *Chothia*'s hypervariable loop H1 extending from positions 26 - 32 should be substituted;
- Secondly, the subject-matter remaining in the claim after inclusion of the disclaimer was considered to be novel and inventive; claim 1 of the first and second auxiliary request were considered to provide a major novel and inventive teaching by reciting 3 specific criteria according to which amino acid substitutions should be made during humanization of immunoglobulins, which criteria are not to be found in the prior art as represented by **D36**. This would appear to be supported by **D40**, which in its conclusive statement as to the work achieved in **D36** (**D40**, page 17/right column/lines 3 - 7 and the last two sentences), defines a still unsolved technical problem; this problem is now claimed to be solved by the method of claim 1. Hence, **D36** was considered to become irrelevant for the purpose of assessing novelty and inventive step after inclusion of the disclaimer.
- Thirdly, the Proprietor argued that the scope of the disclaimer was commensurate in scope with the prior art to be disclaimed as it precisely removed **D36** from coverage, in which document the only amino acid substitutions made by *Riechmann et al.* were outside of *Kabat* CDRs (residues 31 - 35), but inside the first heavy chain hypervariable loop according to *Chothia* (residues 26 - 32), in contrast to the practical implementation of the alleged invention, where at least one amino acid substitution was to be made outside *Chothia* loop H1.



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In this context, the Proprietor pointed to the fact that, on page 18/line 23, the application as originally filed already provided a supporting basis for the disclaimer, as it contained a reference to **D36**.

- Fourthly, following the EPO's usual approach to disclaimers, the prior art itself was considered to provide the relevant supporting basis under Art. 123(2) EPC; claims 1 of the first and second auxiliary request were therefore considered to be in line with Art. 123(2) EPC.

b) As seen by the Opponents:

All Opponents appeared to be in agreement that the *"at least one AA outside Chothia"* feature was unacceptable as a disclaimer and would contravene Art. 123(2) EPC for two main reasons:

- According to established case law such as given in T917/94, T863/96, T963/96, T645/96, a disclaimer is only allowable if the disclaimed document is completely removed from the relevant prior art after inclusion of the disclaimer, that is, if it represents an accidental disclosure. However, the Opponents considered **D36** to represent the closest state of the art available at the priority date of the application as filed. They argued that if the disclaimer would not be present in claim 1, the latter would not be novel and inventive with respect to **D36**, as already acknowledged by the Opposition Division in the preliminary opinion. Hence, the disclaimer would only appear to be included by the Proprietor in claim 1 to make its non-inventive teaching inventive, which clearly contravenes the principles set forth in T917/94 and T170/87.
- The *"at least one AA outside Chothia"* feature is not an appropriate disclaimer, as it does not disclaim the specific embodiment disclosed in **D36**, but a much broader teaching which has been extrapolated by the Proprietor from the specific teaching according to **D36** to render the subject-matter of his claim 1 novel and inventive. The Opponents therefore considered that the disclaimer in claim 1 of the first and second auxiliary request added an additional technical teaching to the claimed



method, which teaching was not derivable from the content of the application as originally filed. The disclaimer was not therefore be considered to be allowable under Art. 123(2) EPC. In this respect, reference was made to T245/91.

c) As seen by the Opposition Division:

In accordance with the case law of the Boards of Appeal, it would be allowable under Article 123(2) EPC to formulate a disclaimer which is precisely defined and limited to the prior art disclosure, provided this disclosure is an accidental novelty-destroying disclosure (T0863/96, paragraph 3.2 of the reasons).

- c1) A disclaimer is only allowable if the prior document containing the excluded disclosure has no relevance for any further examination aspect of the claimed invention; upon introduction of the disclaimer, this prior document must disappear from the prior art field to be taken into consideration (T596/96, paragraph 2.2 of the reasons, and T863/96, paragraph 3.2 of the reasons).

In the present case, document **D36**, allegedly justifying the introduction of the disclaimer "*at least one AA outside Chothia*", indisputably relates to the same field as the claimed invention, namely to the humanisation of an antibody by a CDR grafting method.

Moreover, the examination of all cited prior documents made during substantive examination and opposition phase has already revealed that document **D36**, together with its patent counterpart **D48**, is to be considered to represent the piece of prior art coming closest to the claimed subject-matter, as **D36** attempts to solve the problem of decreased affinity of a particular humanized antibody upon transfer of rat *Kabat* CDRs onto a human framework by substituting additional amino acids in the *Kabat* framework region. Hence, this document remains highly relevant with or without a disclaimer in claim 1. **D36** does not therefore represent an "accidental" anticipation.



The introduction of the "*at least one AA outside Chothia*" feature as a disclaimer into claim 1 does not therefore meet the requirements for allowability of a disclaimer under Art. 123(2) EPC as established by current case law.

c2) Assuming, *arguendo*, that **D36** would indeed be an accidental disclosure, the proposed disclaimer would not be allowable because, firstly, contrary to the Proprietor's assertion, it is not considered by the Opposition Division to have a basis in the application as originally filed and, secondly, it is not precisely defined and limited to the prior art disclosure:

- The reference to document **D36** on page 18/line 23 does not form part of the invention, but is nothing more than an acknowledgement of this document made in the context of preferred genetic engineering techniques. It was certainly not intended to exclude from the scope of the claims the humanized immunoglobulin and method for the production thereof according to **D36**.
- The Opposition Division cannot follow the Proprietor's interpretation that **D36** or its patent counterpart **D48** would disclose a particular approach for the preparation of humanized antibodies, which the Proprietor should be entitled to disclaim in his own claims, in which he clearly would recite a different method.

As best seen in Fig. 1 of **D36**, *Riechmann et al.* disclose the synthesis of a humanized antibody against the CAMPATH-1 antigen, said antibody comprising the CDRs of rat antibody YTH 34.5HL on a human framework (derived from antibody NEW for the heavy chain and antibody REI for the light chain). Intentional Ser27 - --> Phe27 and Ser30 ---> Thr30 framework substitutions were made to restore the helper function in loop packing seen in most human heavy chains, thereby obtaining a significant increase in binding affinity for the CAMPATH-1 antigen in comparison to the humanized antibody having only the CDRs grafted (**D36**, page 326/left column/middle paragraph). As set forth by the Opposition Division in



paragraph **D.3** of its preliminary opinion dated 02/05/99, these 2 substitutions implicitly follow the "3 rules" of claim 1 of the first auxiliary request and claims 1 - 3 of the second auxiliary request. However, the following is noted:

- **D36** discloses the preparation of a specific humanized antibody and there is no explicit universal teaching which the authors conclude could be applicable to the preparation of other humanized antibodies. What the skilled person would learn from **D36** is simply that, in order to increase binding affinity, amino acid residues 27 and 30 of a particular human acceptor framework have been substituted for their rat counterparts. There is simply no indication in **D36** that further amino acid substitutions should be made **outside of a whole region**, as implied by the term "*at least one AA outside Chothia*", which excludes amino acid residues 26 - 32 from the region of the immunoglobulin where "*at least one further amino acid substitution*" should be made.
- In the last sentence on page 326/left column/middle paragraph of **D36**, the authors observe that "... *alterations in the 'Kabat' framework regions can enhance the affinity of the antibody ...*" (underlining added), thereby indicating that their conclusion only applies to the anti-CAMPATH-1 antibody they have prepared. Even the section "Prospects" on page 327 of **D36** only relates to potential therapeutic implications of the same particular humanized anti-CAMPATH1 antibody.
- If some sort of generalized technical lesson can be learned from **D36**, it is that only the *Kabat* framework (hence, the portions of the immunoglobulin outside the *Kabat* CDRs) has been considered for experimental manipulation ("*... alterations in the 'Kabat' framework regions can enhance the affinity of the antibody ...*", underlining added).

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There is no mention whatsoever of any manipulation to be made "... *outside of the first heavy chain hypervariable loop as defined by Chothia et al. [Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)] ...*". The statement given in **D36** on page 326,

"By sequence, the first hypervariable loop extends from residues 31 - 35 (ref. 25 = D27), whereas by structure it extends from residues 26 - 32 (ref. 32 = D28)."

is no more than an acknowledgement of prior art concepts of hypervariable loops, equivalent to what the inventors have done on page 9/line 37 - page 10/line 7 of the application as filed.

In the Opposition Division's view, the disclaimer introduced in claim 1 of the first and the second auxiliary request therefore comprises an unallowable generalization of the teaching according to **D36**.

Moreover, by applying such disclaimer, which does not recite what **D36** teaches, namely 2 amino acid substitutions at positions 27 and 30 in the heavy chain of a humanized anti-CAMPATH1 antibody, to a method for the production of **any** humanized antibody directed against **any** antigen, the Proprietor at the same time excludes from claim 1 a broad area of previously undisclosed subject-matter, and narrows it to technical embodiments which have a basis neither in the application as filed nor in **D36**.

The disclaimer in claim 1 of the first and the second auxiliary request does not comply with Art. 123(2) EPC, because it adds an additional technical teaching to the claimed method which was neither derivable from the content of the application as originally filed nor from **D36**.

C Auxiliary request 3 - Art. 123(2) and (3) EPC

1. Claim 1 of the third auxiliary request substantially corresponds to claim 7 as granted, but includes the subject-matter of claim 10 as granted; hence claim 1 of the third auxiliary request relates to the production of a humanized immunoglobulin, wherein the mature heavy and light variable region sequences of the immunoglobulin obtained by the method have the exact amino acid sequence as given beneath the nucleotide sequences of Figures 3 and 4.

Claims 2 - 13 were adapted correspondingly, with minor modifications in their wording.

2. Opponent 11 challenged the validity of claim 1 of the third auxiliary request under Art. 123(2) EPC by arguing that, owing to the controversial definition of CDRs throughout the application as filed, claim 1 would still cover embodiments which were not originally disclosed. More particularly, he pointed to the fact, that the application as filed would appear to use 2 divergent definitions of the Kabat CDR3 of the heavy chain, said to be composed of amino acid residues 95 - 102 on page 10/lines 3 - 5 (via the reference to *Kabat's* document D15), but being defined as comprising amino acid residues 99 - 106 on page 26/line 26.

Moreover, in the view of Opponent 11, since the Proprietor had always used the combined "*Kabat + Chothia*" definition in his originally filed application (meaning that CDR1 of the heavy chain comprises amino acid residues 26 - 35), and since claim 1 of the third auxiliary request now would appear to relate to *Kabat* CDRs only (meaning that CDR1 of the heavy chain only comprises amino acid residues 31 - 35), the scope of the granted claims has been extended (Art. 123(3) EPC).

3. In reply, the Proprietor merely pointed out that claim 1 of the third auxiliary request was now limited to obtaining, by means of the claimed method, an immunoglobulin having the amino acid sequence as given beneath the nucleotide sequences in Figures 3 and 4, which were already included in the application as originally filed.

4. The Opposition Division fully appreciates the Proprietor's position. Claims 1 - 13 of the third auxiliary request have a clear basis in claims 11, 12, 19 and 22 as well as page 7/lines 20 - 33 and the complete section titled "Experimental" on pages 26 - 32 of the original application; indeed, by limiting its scope to the mature light and heavy chains as depicted in Figures 3 and 4, claim 1 now only covers the preparation of the humanized anti-*Tac* antibody as given in the single illustrative example of the application as filed.

As Figures 3 and 4 were already disclosed in the application as originally filed, claims 1 - 13 of the third auxiliary request clearly meet the requirements according to Art. 123(2) EPC.

Moreover, the scope of protection has not been extended (Art. 123(3) EPC), as claim 1 of the third auxiliary request corresponds to combined claims 7 and 10 as granted, with the additional limitation that the claimed mature light and heavy chain variable region protein sequences are **exactly** as given beneath the nucleotide sequences of Figures 3 and 4, and not "*homologous to*", as recited in granted claim 10.

D Auxiliary request 3 - Art. 84 EPC

1. Some of the Opponents raised objections as to the alleged unclear meaning of the terms "*rare*" and "*predicted*" in criteria (a) and (c) of claim 1. It was also argued that claims 1 - 13 of the third auxiliary request did not meet the requirement of conciseness as recited in Art. 84 EPC, because most of the claim wording was now superfluous with regard to the limitation of claim 1 to the exact amino acid sequences as given beneath the nucleotide sequences of Figures 3 and 4.
2. The Opposition Division agrees with the Proprietor's observation that objections under Art. 84 EPC can only be raised against an amended auxiliary request, if the alleged unclarities were caused by the amendments. Indeed, the terms "*rare*" and "*predicted*" were already used in criteria (a) and (c) of e.g. claim 7 as granted. It is also true that the Opponents have raised Art. 84 EPC objections in their initial notices of opposition against the said terms; however, objections made under Art. 84 EPC do not constitute a ground for opposition, and the Opposition Division



merely wishes to refer to the Proprietor's convincing statements refuting the Opponent's objections, submitted with his first letter dated 07/04/98, page 72/last paragraph - page 74/2nd paragraph.

As concerns the alleged superfluous use of a generic wording in claim 1 to recite a method which would appear to yield exactly one product, it should be emphasized that the Proprietor, by limiting claims 1 - 13 to the immunoglobulin having the amino acid sequence as given beneath the nucleotide sequences of Figures 3 and 4, has only defined the precise structure of the **end product** obtained by using the claimed method. However, the method of claim 1 still has more than one degree of freedom insofar as the choice of **starting products** is concerned. Indeed, the "starting antibodies" are not defined in claim 1, and by selecting different human acceptor and different non-human donor antibodies and applying the 3 criteria for CDR and additional amino acid grafting as set forth in claim 1, one may still arrive at a humanized immunoglobulin wherein the mature light and heavy chain variable region protein sequences are as in Figures 3 and 4 (see also page 17/lines 28 - 35). It should also be borne in mind that claim 1 does not impose structural limits on the sequence of the constant regions of the final immunoglobulin.

The Opposition Division therefore considers the wording used in claims 1 - 13 of the third auxiliary request to be commensurate with the scope of protection sought.

E Auxiliary request 3 - Art. 54 EPC

1. In the view of Opponent 11, since the Proprietor had always used the combined "*Kabat + Chothia*" definition in his originally filed application (meaning that CDR1 of the heavy chain comprises amino acid residues 26 - 35), and since claim 1 of the third auxiliary request now would appear to relate to *Kabat* CDRs only (meaning that CDR1 of the heavy chain only comprises amino acid residues 31 - 35), the latter would not appear to enjoy the priority dates of either the first (PDL1 application US290975, 28/12/88) or second priority application (PDL2 application US310252, 13/02/89). The effective date for the claims of the third auxiliary request would therefore be the filing date 28/12/89.



Hence, document **D52**, allegedly made available to the public on 27/12/89, and being the counterpart of the disputed patent published in the scientific literature, would destroy the novelty of the claims according to the third auxiliary request, as Fig. 2 of **D52** was identical to Figures 3 and 4 of the application as filed.

2. However, in the analysis of entitlement to priority of claim 7 made in its preliminary opinion annexed to the invitation to Oral Proceedings, the Opposition Division has acknowledged that the only instance where the application of criteria (a), (b) and (c) of granted claim 7 for the design of a humanized immunoglobulin has been described, was in the part entitled "EXPERIMENTAL", more particularly pages 21ff of PDL1. This portion of the PDL1 application is **identical** with pages 26 - 32 of the application as filed and relates **exactly** to the preparation of the anti-*Tac* antibody which is now recited (by reference to the amino acid sequences of Figs. 3 and 4) in claim 1 of the third auxiliary request. As this claim 1 substantially corresponds to combined claims 7 and 10 as granted, and since Figs. 3 and 4 of the application as filed correspond to Figs. 1 and 2 of the PDL1 application, it follows that claim 1 of the third auxiliary request enjoys the priority date of the PDL1 application US290975, 28/12/88. **D52** does not therefore belong to the state of the art as defined in Art. 54(2) and (3) EPC and the Opponent's objection are without substance.

As none of the further documents cited by the Opponents discloses immunoglobulins having mature light and heavy chain variable region protein sequences as given beneath the nucleotide sequences of Figures 3 and 4, the subject-matter of claims 1 - 13 of the third auxiliary request is novel.

**F Auxiliary request 3 - Art. 56 EPC**

1. The main objection raised by some of the Opponents was based on document **D11**, which describes the original murine monoclonal anti-*Tac* antibody used in the CDR grafting method of the patent (see page 2/lines 5 - 15 of the application as filed). Later, as it became clear that an IL-2 receptor binding monoclonal antibody such as the anti-*Tac* antibody of **D11** would have considerably useful therapeutic properties, the skilled person wishing to humanize it would come across document **D32** which discloses the basic technique of CDR grafting, i.e. the transfer of non-human CDRs onto a human antibody acceptor framework. When following the additional teaching given by **D32** that, in order to obtain a functional unaltered antibody,

"..., it may be necessary only to transfer those residues which are accessible from the antigen binding site, and this may involve transferring framework region residues as well as CDR residues."

D32, page 7, 4th paragraph

the skilled person would obviously try to replace certain critical amino acids in the human acceptor framework by the corresponding non-human donor amino acids and therefore obtain a humanized immunoglobulin similar to the antibody of claim 1; the latter was not considered by the Opponents to exhibit outstanding or unexpected properties, as the tests described in the patent revealed that it had lost 2/3 of its affinity in comparison with the unaltered murine anti-*Tac* antibody.

2. In reply, the Proprietor pointed to the fact that, by virtue of the limitation of claim 1 to the mature light and heavy chain variable region protein sequences as given beneath the nucleotide sequences of Figures 3 and 4, claim 1 related to a method wherein 15 independent amino acid substitutions had been made in the construction of the particular humanized anti-*Tac* antibody. For the purposes of assessing whether this claim would involve an inventive step, it should be evaluated whether it was obvious to try to replace such a number of independent amino acids in a single antibody according to three criteria which allegedly had been developed by the inventors, taking into account the case law established in numerous decisions of the Boards of Appeal relating to the issue "reasonable



expectation of success" (e.g. in T60/89). He also noted that, if the Opposition Division considered auxiliary requests 1 and 2 to be inadmissible under Art. 123(2) EPC because of the limited teaching given by **D36** (see paragraph **B.,4., c1**) above), the inventors would have needed 15 times the luck the authors of **D36** had, when they obtained a humanized antibody having increased affinity by the substitution of 1 amino acid in the *Kabat* framework.

3. As already set forth above, the subject-matter of claim 1 consists of a method for the preparation of a humanized immunoglobulin, wherein in addition to the step of grafting CDRs from a donor to an acceptor antibody, certain additional amino acids in the acceptor framework are substituted according to criteria (a) - (c), **and wherein the mature light and heavy variable region protein sequences of the thus humanized immunoglobulin have the amino acid sequence as given beneath the nucleotide sequences of Figures 3 and 4.**

Hence, although the wording of claim 1 gives the impression that a generic method is claimed, it is nevertheless limited to the case where an application of the recited method yields a unique immunoglobulin having an exact amino acid sequence in the variable regions of both the heavy and light chain. The said exact definition is based in Figures 3 and 4 which depict the nucleotide sequence of the heavy and light chain variable region gene of the humanized anti-Tac antibody prepared according to the experimental part of the patent, together with the translated amino acid sequence for the part of the gene encoding protein.

The two entities from which the said humanized antibody has been derived, namely the murine anti-*Tac* donor and the human acceptor antibody *Eu*, are shown in Figures 1 and 2. From the combination of Figs. 1/2 with Figs. 3/4, it is clearly recognizable that 12 amino acids at *Kabat* positions 27, 30, 48, 67, 68, 93, 95, 98, 106 - 108 and 110 of the heavy chain and 3 amino acids at *Kabat* positions 47, 59 and 62 have been substituted in the human *Eu* antibody for their murine anti-*Tac* counterparts.

- a) With regard to the purely structural aspect, the nearest prior art document is represented by **D15**, wherein *Kabat* gives the amino acid sequences of both the heavy and the light chain of the human *Eu* antibody. The difference between **D15** and the humanized antibody produced by the method of claim 1 resides in the fact that the CDRs and 15 additional amino acids have been transferred from the murine anti-*Tac* antibody according to **D11**. The said difference accounts for the obtaining of a humanized immunoglobulin still having substantial affinity for its cognate antigen, the IL-2 receptor, while exhibiting negligible immunogenicity in human patients (HAMA response); under its commercial trade name ZENAPAX®, it was the first clinically approved humanized antibody anywhere, proven to exhibit highly desirable therapeutic properties (see e.g. documents **D112**, **D119**, **D120** by the Proprietor).

The objective problem to be solved is therefore the provision of a humanized immunoglobulin having improved therapeutic properties.

The solution provided is the humanized immunoglobulin prepared according to the method of claim 1 of the third auxiliary request, wherein 15 additional amino acids at specific positions in the human heavy and light chain variable regions have been replaced by their murine counterparts.

None of the many documents cited during the opposition phase contributes to the solution provided, as none of them contains a suggestion pointing to the **substitution of exactly 15 amino acids (not 14, not 16) at *Kabat* positions 27, 30, 48, 67, 68, 93, 95, 98, 106 - 108 and 110 of the *Eu* heavy chain and at *Kabat* positions 47, 59 and 62 of the *Eu* light chain (and not at any other position).**

D11, disclosing the murine anti-*Tac* antibody does not mention humanized antibodies, and as best seen from **D40**, an article reviewing the state of the art in the field of antibody engineering in 1988, and referencing e.g. the publications of *Kabat* (**D27**), *Chothia* (**D28**), *Roberts* (**D31**), *Amit* (**D23**), *Verhoeyen* (**D37**) and *Riechmann* (**D36**), none of these publications or the



Winter patent (**D32**) discloses the preparation of a humanized immunoglobulin based on the human *Eu* / murine anti-*Tac* combination.

The subject-matter of claim 1 of the third auxiliary request and claims 2 - 13, being dependent upon or referring to claim 1, therefore involves an inventive step.

- b) Starting from the functional aspect involved in the method of claim 1, that is, only taking into account the mere fact that apart from the CDRs, some extra amino acids have to be transferred from the donor to the acceptor molecule, the nearest prior art document is represented by **D36**, disclosing the synthesis of a humanized antibody against the CAMPATH-1 antigen, said antibody comprising the CDRs of rat antibody YTH 34.5HL on a human framework (derived from antibody *NEW* for the heavy chain and antibody *REI* for the light chain). Intentional Ser27 ---> Phe27 and Ser30 ---> Thr30 framework substitutions were made to restore the helper function in loop packing seen in most human heavy chains, thereby obtaining a significant increase in binding affinity for the CAMPATH-1 antigen in comparison to the humanized antibody having only the CDRs grafted (**D36**, page 326/left column/middle paragraph).

The difference between **D36** and the humanized antibody produced by the method of claim 1 resides in the fact that the latter is based on a human *Eu* / murine anti-*Tac* combination of acceptor / donor antibodies instead of human *NEW* or *REI* / rat YTH 34.5HL as used in **D36** and in that a total of 15 additional amino acids have been transferred from the donor to the acceptor antibody to obtain the desired humanized immunoglobulin, instead of only 2 in the case of the antibody according to **D36**.

As in paragraph a) above, the said difference accounts for the obtention of a humanized immunoglobulin still having substantial affinity for its cognate antigen, the IL-2 receptor, while exhibiting negligible immunogenicity in human patients (HAMA response); under its commercial trade name ZENAPAX®, it has been the first clinically approved humanized antibody anywhere, proven to exhibit highly desirable therapeutic properties (see e.g. documents **D112**, **D119**, **D120** by the Proprietor).



The objective problem to be solved is therefore the provision of a universally applicable set of rules for the reproducible selection of additional amino acid residues to be substituted in the preparation of **any** humanized antibody, in extension of the conclusion reached by the authors of **D36** in the context of a particular humanized immunoglobulin, that

"... alterations in the Kabat framework can enhance the affinity of the antibody."

D36, page 326/left column/last of middle paragraph

The solution provided is the humanized immunoglobulin prepared according to the method of claim 1 of the third auxiliary request, wherein 15 additional amino acids at specific positions in the human heavy and light chain variable regions have been replaced by their murine counterparts, following selection criteria (a) - (c).

None of the many documents cited during the opposition phase contributes to the solution provided, as none of them contains a suggestion pointing to the technical features recited in selection criteria (a) - (c), **the application of such criteria to the humanisation of an anti-*Tac* antibody leading to the substitution of exactly 15 amino acids (not 14, not 16) at *Kabat* positions 27, 30, 48, 67, 68, 93, 95, 98, 106 - 108 and 110 of the *Eu* heavy chain and at *Kabat* positions 47, 59 and 62 of the *Eu* light chain (and not at any other position).**

As already argued in the context of Art. 123(2) EPC, the Opposition Division considers that the 2 substitutions made by *Riechmann et al.* in **D36** implicitly follow at least 2 of the "3 rules" of claim 1; however, **D36** discloses the preparation of a **particular** humanized antibody and there is no explicit universal teaching which the authors conclude could be applicable to the preparation of other humanized antibodies. What the skilled person would learn from **D36** is simply that, in order to increase binding affinity, amino acid residues 27 and 30 of a particular human acceptor framework have been substituted for their rat counterparts. The authors of **D36** were not aware of the fact that their substitution strategy could be formulated in a more generic manner to yield substitution 3 criteria (a) - (c) according to claim 1 of the third



auxiliary request. Hence, they neither proposed that a similar strategy could be used when attempting to humanize other antibodies, nor that further amino acid substitutions deep in the *Kabat* framework should be made.

While it is true that the publications of Chothia (D28), Roberts (D31), Amit (D23), Verhoeyen (D37), Cheetham (D40) and Winter (D32), in the wider context of engineering the antigen binding site and maintaining or improving antibody affinity, identify potential problems with some particular amino acids in the *Kabat* framework, none of them provides a universal solution by formulating substitution rules similar to the 3 criteria (a) - (c) of claim 1 and suggesting that such rules could be employed in antibody humanisation.

Most importantly, none of the cited documents suggests that **exactly 15 amino acids (not 14, not 16) at *Kabat* positions 27, 30, 48, 67, 68, 93, 95, 98, 106 - 108 and 110 of the *Eu* heavy chain and at *Kabat* positions 47, 59 and 62 of the *Eu* light chain (and not at any other position)** should be made in order to successfully apply the method as defined by criteria (a) - (c) to the preparation of the humanized anti-*Tac* antibody of claim 1.

The subject-matter of claim 1 of the third auxiliary request and claims 2 - 13, being dependent upon or referring to claim 1, therefore involves an inventive step.



G Rejection of the Proprietor's request to submit another auxiliary request during oral proceedings

In view of the complexity of the case and the number of parties involved, the Opposition Division, in its preliminary opinion annexed to the invitation to attend Oral Proceedings, has set a time limit of 3 months to make written submissions in preparation of the oral proceedings (Rule 71a(1) EPC). On 17/12/99, i.e. 3 days before the expiry of the above time limit, the Proprietor has filed 5 auxiliary requests, together with new supporting documents **D112 - D129**.

Clearly, the Opponents did not have time to file their comments on the 5 auxiliary requests within the time limit set under Rule 71a(1) EPC, but the Opposition Division considered that the period of 3 months still available before the date of the Oral Proceedings would provide them with a fair opportunity to prepare such comments for oral submission.

Nevertheless, further substantial observations, partly on the preliminary opinion issued by the Opposition Division and partly on the letter of the Proprietor dated 17/12/99, were submitted by Opponent 4 on 16/12/99, the submission introducing further documents **D130 - D134** as listed in Annex A, and Opponent 8 on 17/12/99, 08/03/00 and 13/03/00, the submission introducing further documents **D135 - D141** as listed in Annex A.

With a letter dated 16/03/00 (that is, **4 days before the date of the oral proceedings**), the Proprietor submitted a new set of 3 auxiliary requests, wherein auxiliary requests 1 and 2 were derived from auxiliary requests 3 and 4 as filed on 17/12/99 and auxiliary request 3 was identical with auxiliary request 5 as filed on 17/12/99. The submission was said to be caused by an effort to overcome a novelty objection made by Opponent 8 and being based on an alleged early publication date of document D52.

At this point of the proceedings, and although the Proprietor asserted that a simplification of the procedure had been obtained by the reduction in the number of auxiliary requests, the amendments carried out in the wording of the claims (such as the replacement of "framework" by "**Kabat framework**") were already



considered by the Opposition Division to place a heavy burden on the Opponents insofar as they possibly had to substantially modify their argumentation shortly before the oral proceedings, taking into account that the meaning attributed to terms such as "*Kabat CDRs*", "*Kabat framework*" or "*Chothia loops*" were absolutely critical in the evaluation of the merits of the contested patent.

Nevertheless, in the opening phase of the Oral Proceedings, none of the Opponents lodged a protest against the acceptance by the Opposition Division of late filed auxiliary requests 1 - 3.

After rejection of the main request as well as auxiliary requests 1 and 2 for non-compliance with Art. 123(2) EPC, the Proprietor requested the Opposition Division to set forth the exact reasons for the rejection; he furthermore requested an opportunity to file another auxiliary request which would take into account the said reasons. The Proprietor argued that he could not have reasonably have foreseen that auxiliary requests 1 and 2 would be rejected under Art. 123(2) EPC.

In reply, some of the Opponents announced that, should the Opposition Division be inclined to accept the Proprietor's requests, they would seek an adjournment of the Oral Proceedings and an award of costs.

The Opposition Division decided to refuse the Proprietor's request for filing another auxiliary request **as being a late filed request**, for the following reasons:

- By having filed his first set of 5 auxiliary requests shortly before expiry of the time limit set under Rule 71a(1) EPC, the Opponents were deprived of the opportunity to file comments thereon within the said time limit.
- The Proprietor had sufficient opportunity to file auxiliary requests suitable to overcome the grounds of opposition put forward by the Opponents. Acceptance of the last 3 auxiliary requests filed 4 days before the oral proceedings by the Opposition Division (and apparently also by the Opponents) is already considered to constitute a benevolently granted supplementary opportunity for the Proprietor to defend his position.


**Entscheidungsgründe (Anlage)****Grounds for the decision (Annex)****Motifs de la décision (Annexe)**

Datum
Date
Date
14.02.2001

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Anmelde-Nr.:
Application No.:
Demande n°:
90 903 576.8

- No further request was presented at the opening phase of the Oral Proceedings.
- It is neither usual in Oral Proceedings for an Opposition Division to proclaim the exact reasons for the rejection of a particular request nor is it common practice to grant a party sufficient time to formulate an auxiliary request which takes into account each and every such reason in order to finally obtain an allowable request overcoming all objections raised.
- For reasons of fairness, the Opponent's request for postponement of the Oral Proceedings, should the Proprietor's request be allowed, would have to have been accepted, in order to give them the necessary time to study the amendments proposed. With regard to the number of Opponents involved, such a postponement would have caused an unacceptable delay of the procedure.
- After rejection of the first and second auxiliary requests, there was still a third auxiliary request on the table which had not been discussed. Hence, the rejection by the Opposition Division of the previous auxiliary requests did not automatically lead to the revocation of the patent.

	Entscheidungsgründe (Anlage)	Grounds for the decision (Annex)	Motifs de la décision (Annexe)
	Datum Date Date 14.02.2001	Blatt Sheet Feuille 49	Anmelde-Nr.: Application No.: Demande n°: 90 903 576.8

III. Decision

- For the reasons given above, the Opposition Division considers that neither claim 1 of the main request, nor claim 1 of the first or claim 1 of the second auxiliary request meet the requirements according to Art. 123(2) EPC.
- *Obiter dictum*, the disclaimer introduced in claim 1 of the first and claim 1 of the second auxiliary request, "*at least one AA outside Chothia*" does not comply with the established case law of the EPO's Boards of Appeals on the allowability of disclaimers..
- Claims 1 - 13 of the third auxiliary request have a clear basis in the application as originally filed; their scope has not been extended over the scope of the claims as granted; the said claims therefore comply with the requirements according to Art. 123(2) and (3) EPC.
- Claims 1 - 13 of the third auxiliary request meet the requirements according to Art. 84 EPC.
- Claims 1 - 13 of the third auxiliary request meet the requirements according to Art. 54 EPC, since their respective subject-matter is not disclosed in the available prior art documents.
- Claims 1 - 13 of the third auxiliary request meet the requirements according to Art. 56 EPC, since their respective subject-matter is not obviously derivable from any of the available prior art documents, taken alone or in combination.
- The Proprietor's request for filing a further auxiliary request during oral proceedings is refused.

According to Art. 102(3) EPC, the patent is therefore maintained on the basis of claims 1 - 13 of the third auxiliary request, pages 1, 2, 4, 6 and 8 - 13 of the patent specification, pages 3, 5 and 7 of the adapted description as filed with the letter dated 12/06/00, and Figures 1 - 10 of the patent specification.

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June 22, 2001
Jae/PST/thc

We refer to our appeal filed on April 20, 2001 against the decision of the Opposition Division dated February 14, 2001 in the above-referenced case. Please find enclosed 21 copies of our new Main Request and of two Auxiliary Requests. Please note that Hepworth, Lawrence, Bryer & Bizley and Mr. Bizley specifically remain the address for service in this case. It is therefore respectfully requested that the European Patent Office should continue to direct all formal correspondence and copy correspondence to Mr. Richard Bizley. Furthermore, we substantiate our appeal as follows:

INTRODUCTION

The invention claimed in the patent relates generally to the combination of recombinant DNA and monoclonal antibody technologies for the production of

non-immunogenic antibodies and their uses. The methods of the claimed invention for the first time enabled a general approach to providing improved forms of humanized antibodies that exhibit satisfactory binding capabilities while remaining substantially non-immunogenic in humans.

The prior art methods for producing humanized antibodies relied upon the presumed "self-correcting" of the framework in the assembly of the CDRs on binding of the antigen; see, for example, Verhoeyen (D37) and Verhoeyen (D35). The work of Riechmann (D36) raised questions as to what extent the framework of a given antibody can provide a passive scaffold onto which the CDRs from another antibody may be grafted and retain their antigen binding affinity. Although in Riechmann (D36) antigen binding could be improved by particular considerations on the specific antibody investigated, no conclusion could be reached on how to generally approach antibody humanization when facing substantial loss of binding affinity upon CDR grafting only. Accordingly, when summarizing the prior art, Cheetham (D40) concluded with emphasis that "[m]odifying the properties of immunoglobulin molecules may after all prove to be more a matter of 'tinkering' by molecular biology, than a tidy 'tailoring' of CDRs by the antibody engineer!"; see Cheetham (D40) on page 172, right column, last sentence. Thus, literally speaking according to Cheetham (D40), the person skilled in the art in 1988 when trying to improve CDR grafted antibodies would have no choice other than tinkering, i.e. to work unskillfully and without guidance at the improvement of CDR grafted antibodies by trial and error on a case by case basis.

In contrast, the invention claimed in the patent is based on the inventive concept that for producing humanized antibodies, the acceptor framework has to be adjusted to match the framework of the CDR donor antibody according to certain rules, in order to substantially retain the binding affinity of the donor antibody. Thus, the claimed invention provides a method for the production of humanized antibodies, wherein one or more amino acid(s) in the human acceptor framework are substituted by corresponding amino acids from the framework of the donor

antibody according to certain criteria. The claimed methods have been proven to be generally applicable and to allow the successful humanization of antibodies in numerous publications; see the documents submitted with our observations, dated April 7, 1998 and December 17, 1999. As explained in the overview at page 1 of the latter observations, the anti-Tac antibody produced in accordance with the claimed invention was the first clinically approved humanized antibody anywhere in the world. Furthermore, several humanized antibodies produced in accordance with the claimed method have now been approved for marketing, and more than 30 are currently in clinical trials. Thus, with the rules given in the claims and in the patent, the person skilled in the art no longer has to tinker by trial and error, but now has the general means and methods to tailor improved forms of humanized antibodies with therapeutic utility.

1. GENERAL REQUESTS

We request to set aside the decision of the Opposition Division dated February 14, 2001 and to maintain the above-identified patent on the basis of our new Main Request, 21 copies of which are enclosed herewith.

As a precautionary measure only, we enclose herewith two auxiliary requests. Furthermore, we reserve the right to file further auxiliary requests in case the Technical Board feels minded to object to our Main Request.

In order to expedite the proceedings, we request that an intermediate notice be issued by the Technical Board giving its preliminary opinion on the matter once it has considered the first written submissions of all parties.

As a precautionary measure, oral proceedings in accordance with Article 116 EPC are requested.

2. NEW CLAIMS REQUESTS

2.1 New Main Request

2.1.1 Claim 1

Claim 1 in the Main Request corresponds to claim 7 as granted but differs therefrom in that it contains a a final disclaiming clause at the end of the claim. The latter removes from coverage any accidental inclusion within the scope of the claim of the teachings of EP-A 0 328 404 (D48), the disclosure of which is virtually identical to that of Riechmann (D36) with respect to the matter relevant for the claim.

The wording of the disclaimer is based on the disclosure content of EP-A 0 328 404 (D48) inter alia at page 3, lines 31 to 36; Figure 2a and claims 8 and 9. Since EP-A 0 328 404 (D48) always refers to the antibody, i.e. the HuVHCAMP antibody shown in Figure 2 (hereinafter also referred to as the humanized anti-Campath-1 antibody), the disclaimer is commensurate in scope with the matter to be excluded from the subject matter of claim 1.

The reference to the published patent application is also appropriate and the most efficient way to formulate the disclaimer rather than for example to incorporate the respective amino acid sequences. Such wording of a disclaimer has been allowed in previous cases in the EPO; see for example European patent EP-B1 0 142 924 subject of T 116/95 "Insect resistant plants/MYCOGEN", dated April 26, 1999, not yet published in the OJ EPO.

2.1.2 Claims 2 to 15

Claims 2 to 15 correspond to claims 8 to 10 and 12 to 21 with back-references adjusted.

2.2 **Auxiliary Request I**

2.2.1 Claim 1

Claim 1 of Auxiliary Request I corresponds to granted claim 7, wherein the subject matter of granted claim 8 has been incorporated.

2.2.2 Claims 2 and 3

Claims 2 and 3 of the Auxiliary Request correspond to granted claims 9 and 10 with back-references adjusted.

2.2.3 Claim 4

Claim 4 corresponds to granted claim 12 but specifically recites the immunoglobulin heavy chain.

2.2.4 Claim 5

Claim 5 is based on the subject matter of granted claim 12 wherein the humanized immunoglobulin light chain has been defined as being obtainable by a method according to claim 3.

2.2.5 Claim 6

Claim 6 corresponds to granted claim 13 with the amendment that the reference to the light chain has been defined by being obtainable by a method of any one of new claims 1 to 3.

Since in granted claim 13 the light chain is defined by reference to granted claim 12 which in turn defined the immunoglobulin chains as being obtained by the method of any one of granted claims 7 to 10, the amendment is fully in line with the granted claims and does not extend the scope of claim 6 beyond that of corresponding granted claim 13.

2.2.6 Claims 7 to 14

Claims 7 to 14 correspond to granted claims 14 to 21 with back references adjusted.

2.3 **Auxiliary Request II**

2.3.1 Claim 1

Claim 1 of Auxiliary Request II corresponds to granted claim 7 but has been directed to the production of a humanized immunoglobulin light chain.

2.3.2 Claims 2 to 4

Claims 2 to 4 correspond to granted claims 8 to 10.

2.3.3 Claim 5

Claim 5 corresponds to granted claim 12 in combination with granted claim 10 and refers to the immunoglobulin light chain..

2.3.4 Claim 6

Claim 6 relates to a humanized immunoglobulin heavy chain and is based on the subject matter of granted claim 12 in combination with granted claim 10.

2.3.5 Claims 7 to 15

Claims 7 to 15 correspond to granted claims 13 to 21 with back-references adjusted.

2.4 **The Claims Requests comply with the requirements of Article 123(2), 123(3) and 84 EPC**

Since the claims of the Main and the Auxiliary Requests are solely based on the combination of granted claims 7 to 10 and 12 to 21, they necessarily do not extend the scope of the granted claims and are supported by the application as filed for the same reasons as the granted claims. Therefore, the claims of the main and auxiliary requests comply with the requirements of Article 123(3) and 123(2) EPC. Furthermore, since no amendments have been made to the terms in the claims, the requirements of Article 84 EPC are met as well.



3. THE NEW MAIN REQUEST DOES NOT ADD MATTER

3.1 The meaning of the term "CDRs" in granted claim 7 and in the new Main and Auxiliary Requests

In its conclusion as to Article 123(2) EPC concerning the definition of CDRs in the claims as granted, the Opposition Division on page 27, section A.7 of the written decision stated that granted claim 7 would imply the CDR definition given in granted claim 1 and therefore did not meet the requirements of Article 123(2) EPC. Furthermore, in the following section, the Opposition Division stated that reverting to the commonly accepted "Kabat" CDR definition would result in an unallowable broadening under Article 123(3) EPC.

Patentee disagrees with the Opposition Division's assessment of granted claim 7 and its dependent claims.

First of all, as acknowledged by the Opposition Division on page 24 of its written decision, section A.d6, the application as filed is in perfect agreement with Kabat's CDR definition. This is also in line with the examples of the patent which are intended to illustrate the invention.

Moreover, as argued vigorously by the Opponents and accepted by the Opposition Division, the Chothia "definition" of CDR is non-standard, and not widely used by skilled persons. Thus, unless specifically defining CDR's otherwise as done in granted claim 1, the person skilled in the art when reading the application as filed and the patent specification would have inevitably understood that the CDRs in granted claim 7 referred to Kabat CDRs.

Second, as explained in section A.2 of Patentee's observations dated April 7, 1998, the invention can be expressed in a number of ways. Thus, in granted claim 1 Patentee attempted to define the contribution

of the invention as substituting one or more amino acids in the antibody variable region with corresponding amino acids from the variable region of the non-human donor antibody in addition to transferring the CDRs or hypervariable regions taking into account, as in Cheetham (D40) and Riechmann (D36), the impact of Kabat (D27) and Chothia (D28). Accordingly, in granted claim 1 the CDRs have been specifically defined as comprising the definition of hypervariable regions by both Kabat (D27) and Chothia (D28).

Alternatively, however, Patentee defined the method of the invention in terms of transferring the CDRs together with substituting from the framework region of the donor antibody amino acids that meet the important criteria as set out in features (a) to (c) in granted claim 7. In this aspect, the criteria or rules given in granted claim 7 provide the novel and inventive teaching, which has been particularly illustrated in the examples with the Kabat CDRs. The term "framework region" used twice in granted claim 7 is unambiguously defined as the portion other than the Kabat CDRs in the patent specification on page 5, lines 50 to 53 corresponding to page 10, line 37 to page 11, line 3 of the application as filed. Correspondingly, the term CDR used in this context must necessarily mean Kabat CDR. Please note that the term "framework region" was not used in granted claim 1 and therefore allowed the definition of CDRs or hypervariable regions by Kabat (D27) and Chothia (D28) in granted claim 1. However, the CDRs in granted claim 7 have never been and never were intended to refer to CDRs other than those as defined by Kabat (D27). Therefore, the term "framework region" was purposely used to indicate this.

Furthermore, granted claim 7 is an independent claim and therefore has to be assessed independently from granted claim 1. In fact, in its preliminary opinion attached to the summons of May 12, 1999 the Opposition Division in section B.3 at page 16 explicitly stated that

"for the purpose of analyzing claim 7 ... this term [CDRs] must and will be interpreted in the commonly accepted technical sense, i.e. *Kabat's* definition of hypervariable region based on sequence variability and therefore meaning residue 31-35 for CDR1 of the heavy chain"

Accordingly, the Opposition Division in its preliminary opinion came to the conclusion that claim 7 as granted met the requirements of Article 123(2) EPC. It is quite surprising and unclear how the Opposition Division in its written decision came to just the opposite conclusion, in particular since none of the Opponents contested the Opposition Division's preliminary opinion on the term CDRs in granted claim 7 in their last written submissions. To the contrary, they heavily argued that the term CDRs in granted claim 7 could only mean *Kabat* CDRs and therefore that granted claim 7 lacked novelty over Riechmann (D36).

Indeed, it appears as if the Opposition Division's consideration as expressed in sections A.7 and A.8 with respect to granted claim 7 has only arisen by writing the decision, since otherwise the Opposition Division would have necessarily found the Third Auxiliary Request upon which the patent was maintained inadmissible under Article 123(2) and/or 123(3) EPC. This is because while the product directly obtained by the method of claim 1 of the 3rd Auxiliary Request, i.e. the humanized anti-Tac antibody is not affected, applying the Opposition Division's conclusion regarding the definition of CDRs in granted claim 7, the method per se would of course be affected if the meaning of the term CDRs indeed changed. This is even acknowledged by the Opposition Division in the first full paragraph on page 38 of its written decision.

Since the meaning of the term CDR has not changed in claim 1 of the Third Auxiliary Request when combining the subject matter of granted

claims 7 and 10, it can only mean that at the oral proceedings the Opposition Division must have interpreted the term CDRs in granted claim 7 as meaning Kabat CDRs since otherwise it could never have been acknowledged that the Third Auxiliary Request met the requirements of Article 123(2) EPC and 123(3) EPC. Accordingly, the statement in sections A.7 and A.8 on page 27 of the written decision must be in error.

In summary, it is Patentee's position that the term CDRs in granted claim 7 and therefore in claim 1 of the new Main Request refers and always referred to Kabat CDRs.

3.2 The disclaimer in claim 1 of the New Main Request is in line with the requirements of the EPO case law

3.2.1 The requirements for admissibility of a disclaimer in the EPO

The first Technical Board's decision dealing with a disclaimer was T 4/80, "Polyether polyols/BAYER", OJ EPO 1982, 149. In Headnote I the Board generally stated:

"I. Originally disclosed subject-matter clearly definable by technical features, may, at the applicant's request, be excluded from a wider claim by a disclaimer, if the subject-matter remaining in the claim cannot technically be defined directly (positively) more clearly and concisely."

As explained in section 3 of the reasons, the only requirement for a disclaimer is its compliance with Article 84 EPC. This approach was and is also consistent with the practice of EPC contracting states, for example that of the UK and Germany.



In the second landmark decision T 433/86, "Modified Diisocyanat compositions/ICI", dated December 11, 1987, not published in the OJ EPO, the Board held:

"when there is an overlap between the prior art and the claimed subject-matter defined in generic terms, a specific prior art may be excluded even in the absence of support for the excluded matter in the original documents. Such an exclusion may be achieved by way of a disclaimer, or preferably in positive terms if this leads to a clearer and more concise language" (cf. Decision T 04/80, 'Polyetherpolyols/Bayer', OJ EPO 4/1982, 149); see T 433/86 at section 2. (emphasis added)

The third leading decision T 170/87, "Hot-gas cooler/SULZER", dated 5th July 1988, OJ EPO 1989, 441, confirms this principle. It is true that Headnote II of T 170/87 states that a disclaimer cannot make an obvious teaching inventive. However, as explained by the Board in the reasons of the decision, a practical need for disclaimer exists and a disclaimer is admissible as long as the remaining subject matter is inventive. Thus, the Board explained at sections 8.4.1 to 8.4.3:

"8.4.1 According to established Board of Appeal case law, in cases where what is claimed in general overlaps with the state of the art it is permissible to exclude a special state of the art from the claimed invention by means of a disclaimer, even if the original documents give no (specific) basis for such an exclusion" (cf. Decision T 04/80, "Polyether polyols/BAYER", OJ EPO 1982, 149; also, for example, T 433/86 dated 11 December 1987, unpublished, especially point 2).

8.4.2 In Decision T 313/86 dated 12 January 1988 (unpublished) the Board also stated that the same principles apply when a smaller partial area of the generally defined subject-matter of the invention is to be excluded not in view of the state of the art but because it does not solve the existing technical problem (sub-section 3.5, pages 8 to 9, of the aforementioned decision).



8.4.3 The practice referred to in the two preceding sub-sections is justified on the basis of the following considerations: The inventive teaching originally specifically disclosed in the application is not changed as a whole merely by delimiting it with respect to the state of the art or with respect to what has proved not to be functional; rather through the disclaimer (or through a "positive" wording leading to the same result), only the part of the teaching which the applicant cannot claim owing to lack of novelty or reproducibility is "excised" in the sense of a partial disclaimer. A considerable practical need for this exists. All that is necessary is to define appropriately what under the given circumstances is left of the inventive teaching originally disclosed that is still capable of being protected." (emphasis added)

Hence, this decision, which is often relied upon by the Technical Boards of Appeal in recent decisions, provides no basis for the requirement that the prior art to be removed from coverage must be "accidental" and no longer relevant for the assessment of inventive step. To the contrary, the concept and even need of disclaimers for protecting an inventive teaching is confirmed.

Regarding the requirements of Article 123(2) EPC, the Enlarged Board of Appeal G 1/93, "Limiting feature/ADVANCED SEMICONDUCTOR", OJ EPO 1994, 541 held in section 16 of the reasons that the addition of an undisclosed feature limiting the scope of protection conferred by a patent, i.e. a disclaimer, does not violate Article 123(2) EPC if the feature in question merely excludes protection for part of the subject-matter of the claimed invention as covered by the application as filed and does not provide a technical contribution to the claimed subject-matter.

Accordingly, the requirements for the admissibility of the introduction of a disclaimer in light of the leading decisions T 4/80, T 433/86 and T 170/87 and G 1/93 are that:



- (1) the claimed subject-matter cannot technically be defined positively more clearly and concisely;
- (2) the disclaimer limits the scope of protection of the original and granted claims; and
- (3) the feature of the disclaimer does not provide a technical contribution to the claimed subject-matter.

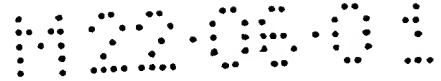
Accordingly, the Guidelines for Examination in the European Patent Office clearly state that an element clearly defined by technical features may be expressly excluded from the protection claimed, for example, in order to meet the requirement of novelty; see the Guidelines part C, Chapter III, section 4.12.

As we will show in the following, the above conditions are met by our new Main Request.

3.2.2 Patentee is entitled to disclaim the subject matter relevant under Article 54(3)(4) EPC of the earlier patent application EP-A 0 328 404 (D48)

EP-A 0 328 404 (D48) was filed on February 10, 1989, claiming the priority of two earlier British applications of February 12 and 25, 1988. Since the application was published only after the relevant second priority date of the present patent but has an earlier filing date, it constitutes prior art under Article 54(3) (4) EPC. It therefore is relevant for the assessment of novelty only and not for inventive step.

EP-A 0 328 404 (D48) teaches the preparation of the humanized anti-Campath-1 antibody, wherein in the heavy chain of the acceptor framework substitutions are to be made at positions 27 or 30 or both; see also section 2.1.1, supra. These two amino acid substitutions incidentally happen to meet the criteria of the method of granted claim 7, i.e. claim 1 of the new Main Request. Since it is not possible to positively distinguish the new and inventive method of claim 1 of the



new Main Request from the incidental disclosure in EP-A 0 328 404 (D48), the relevant subject matter from this application has been excluded from the protection by means of a disclaimer in accordance with the rationale as set forth, for example, in T 433/86.

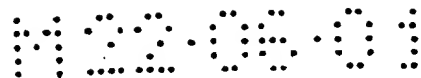
First, as already discussed in section 2.1.1, supra, the subject matter of the disclaimer is supported by and exactly corresponds to the relevant disclosure in EP-A 0 328 404 (D48). Furthermore, the disclaimer has been formulated in a clear and concise manner.

Second, the disclaimer clearly limits the scope of protection of the original and granted claims.

Third, in view of the fact that the disclaimer merely removes from coverage a particular embodiment incidentally happening to fall within the scope of granted claim 7, the subject matter of the disclaimer is not a feature that provides a technical contribution to the claimed subject matter.

Fourth, since EP-A 0 328 404 (D48) is citable only for novelty, once the claimed subject matter has been delimited from the relevant disclosure of this document it is no longer relevant.

Fifth, although a scientific publication Riechmann (D36) corresponding to EP-A 0 328 404 (D48) exists, it is entirely appropriate to formulate a disclaimer on the basis of EP-A 0 328 404 (D48) rather than on the basis of Riechmann (D36). This is because Riechmann (D36) describes only two embodiments which incidentally fall within the scope of claim 1, i.e. a heavy chain with a substitution at position 27 and a heavy chain with substitutions at positions 27 and 30. In contrast, due to, for example, the wording of claims 8 and 9, EP-A 0 328 404 (D48) discloses in addition a heavy chain with the substitution at position 30



only. Therefore, the relevant disclosure in EP-A 0 328 404 (D48) is broader than that of Riechmann (D36). It is therefore all the more justified to base the disclaimer on the disclosure of EP-A 0 328 404 (D48).

Furthermore, it has been the continuous practice of the EPO to generally allow disclaimers for subject matter disclosed by an earlier application under Article 54(3)(4) EPC; see for example Singer/Stauder, EPÜ Artikel 84, section 19-21 "4. Disclaimer". If thereby the disclosure content of Riechmann (D36) is inevitably excluded as well, this does not harm the allowability of the disclaimer for the disclosure of EP-A 0 328 404 (D48). However, even if account is taken of the fact that Riechmann (D36) is prior art under Article 54(1)(2) EPC, this does not change the conclusion that the disclaimer in the new Main Request meets the requirements of the EPC.

3.2.3 The disclaimer in claim 1 is commensurate with the teaching of EP-A 0 328 404 (D48) and of Riechmann (D36)

As mentioned in section 3.2.2, supra, EP-A 0 328 404 (D48) and the corresponding scientific publication Riechmann (D36) disclose the preparation of the humanized anti-Campath-1 antibody, wherein in the heavy chain of the acceptor framework substitutions have been made at positions 27 and/or 30; see also the discussion of Riechmann (D36) and EP-A 0 328 404 (D48) in sections F.1.1 and F.1.4 respectively of Patentee's observations dated April 7, 1998, and the Opposition Division's characterization of Riechmann (D36) in its written decision. These two amino acid substitutions incidentally happen to meet the criteria of the method of claim 7 as granted, i.e. claim 1 of the new Main Request.



Nowhere in EP-A 0 328 404 (D48) and Riechmann (D36) is there disclosed or suggested the substitution of an amino acid residue in the Kabat-framework other than residues 27 and 30 for the particular HuVHCAMP antibody. This has also been acknowledged by the Opposition Division on page 34 of its written decision where it is stated:

"D36 discloses the preparation of a specific humanized antibody and there is no explicit universal teaching which the authors conclude could be applicable to the preparation of other humanized antibodies. What the skilled person would learn from D36 is simply that, in order to increase binding affinity, amino acid residues 27 and 30 of a particular human acceptor framework have been substituted for their rat counterparts. ...

In the last sentence of page 236/left column/middle paragraph of D36, the authors observe that '*... alterations in the 'Kabat' framework regions can enhance the affinity of the antibody ...*' (underlining added), thereby indicating that their conclusion only applies to the anti-CAMPATH-1 antibody they have prepared. Even the section "Prospects" on page 327 of D36 only relates to potential therapeutic implications of the same particular humanized anti-CAMPATH1 antibody;" (emphasis added in the first paragraph)

In fact, the disclaimer has now been worded as requested by the Opposition Division on page 35 of its written decision. That is to "recite what D36 teaches, namely 2 amino acid substitutions at positions 27 and 30 in the heavy chain of a humanized anti-CAMPATH1 antibody"; see the penultimate paragraph on page 35.

3.2.4 EP-A 0 328 404 (D48) and Riechmann (D36) do not provide a solution to the problem

There is not any indication in EP-A 0 328 404 (D48) and Riechmann (D36) which could suggest a solution to the envisaged technical problem, viz. that the affinity of a given humanized antibody for therapy could be substantially improved by employing substitutions of amino

acid residues in the Kabat framework according to the rules of claim 1. Indeed, according to the Opposition Division, involvement of an inventive step would have to be recognized. Hence, on page 44 and 45 of its written decision the Opposition Division stated:

"**D36** discloses the preparation of a **particular humanized antibody** and there is no explicit universal teaching which the authors conclude could be applicable to the preparation of other humanized antibodies. What the skilled person would learn from **D36** is simply that, in order to increase binding affinity, amino acid residues 27 and 30 of a particular human acceptor framework have been substituted for their rat counterparts. The authors of D36 were not aware of the fact that their substitution strategy could be formulated in a more generic manner to yield substitution 3 criteria (a) – (c) according to claim 1 of the third auxiliary request. Hence, they neither proposed that a similar strategy could be used when attempting to humanize other antibodies, nor that further amino acid substitutions deep in the *Kabat* framework should be made.

While it is true that the publications of Chothia (**D28**), Roberts (**D31**), Amit (**D23**), Verhoeyen (**D37**), Cheetham (**D40**) and Winter (**D32**), in the wider context of engineering the antigen binding site and maintaining or improving antibody affinity, identify potential problems with some particular amino acids in the *Kabat* framework, none of them provides a universal solution by formulating substitution rules similar to the 3 criteria (a) – (c) of claim 1 and suggesting that such rules could be employed in antibody humanisation"; see the paragraph bridging pages 44 and 45 and the following paragraph. (emphasis in bold by the Opposition Division)

For the time being, let it be observed that the teaching of Riechmann (D36) and the corresponding application EP-A 0 328 404 (D48) does not contain any suggestions going beyond what has been actually disclosed and disclaimed from the scope of claim 1. This is also evident from the review article "Engineering of antibodies" (D35) authored by Dr. Riechmann together with another renowned scientist, Dr. Martine



Verhoeeyen, author of Verhoeeyen (D37). Though these two leading scientists in the field had access to all relevant art at the priority date of the Patent and had even more information at hand than was available to the general public, they did not suggest, let alone disclose, the possibility of humanizing antibodies by altering framework amino acids according to the rules of the Patent. In this respect, we would like to note that in contrast to usual publications, review articles like that of Drs. Verhoeeyen and Riechmann (D35) are not subject to a reviewing process by referees but are an opportunity to present speculation and outlook for future embodiments. Drs. Riechmann and Verhoeeyen made it very clear what for them was possible in the field of antibody engineering. Figure 3 of (D35) represents their imagination of possible novel versions of antibodies. At the top of this figure it is even emphasized to the person skilled in the art what at that time was thought to be possible: "only CDRs are murine"! It can hardly be true that the average person skilled in the art should be able to conceive more than what was thought and disclosed to be possible by two leading scientists in this field.

3.3 The recent case law is not applicable to the present case

Regarding the case law of the Boards of Appeal on the allowability of disclaimers, the Opposition Division in section B.3.c1 on page 32 of the written decision referred to two recent decisions of the Technical Board 3.3.2, T 863/96 and T 596/96. In view of the reasoning in these two decisions, the Opposition Division came to the conclusion that the case law of the Boards of Appeal may not permit a disclaimer in the present case because Riechmann (D36), the disclosure of which is inevitably removed by the disclaimer of (D48), would still be the "piece of prior art coming closest to the claimed subject matter"; see last paragraph on page 32 of the written decision. However, we respectfully submit that this statement is contrary to the facts and the Opposition Division's own assessment of the teaching of Riechmann (D36).



3.3.1 The Opposition Division's interpretation of the case law regarding the admissibility of disclaimers was not correct

The decisions T 863/96 and T 596/96 as interpreted and applied by the Opposition Division would be in conflict with the interpretation of the EPC by other Appeal Boards and therefore give rise to important general questions on the uniform application of the law and corresponding national law of the EPC contracting states. In this context we would be happy to provide a summary of the EPO case law, which will show that disclaimers have always been permitted when their objective was to distinguish over the prior art disclosure and where no positive features are available to define the remaining subject matter more clearly and concisely; see also section 3.2.1, supra. Thus, if the disclaimer was clear under Article 84 EPC, had a basis in the prior art to be removed, and the scope of the claims was restricted without changing the nature of the originally claimed invention, it has been the continuous practice of the Technical Boards of Appeal to allow the introduction of a disclaimer. As mentioned before, this is also consistent with jurisprudence in the contracting states of the EPC.

In any case, both cases referred to by the Opposition Division have their own merits and are not applicable to the present situation.

In T 0863/96-3.3.2, "Deprenyl/SOMERSET PHARMACEUTICALS", dated February 4, 1999, not yet published in the OJ EPO, the Board actually did not need to take a decision on the allowability of a disclaimer. It merely expressed its doubts because the prior art document dealt with the same drug of the there claimed invention and its use for the same therapeutic indication; see section 3.2 of T 0863/96.



In the fact situation underlying T 0596/96-3.3.2, "Pro-liposome/PHARES PHARMACEUTICAL", dated December 14, 1999, not yet published in the OJ EPO, a first condition for the allowability of the introduction of a disclaimer, i.e. that the prior art document is indeed novelty-destroying, had not been met.

Thus, the fact situations underlying the mentioned decisions considerably differ from the present case.

Regarding the general applicability of the catchword of T 863/96 quoted by the Opposition Division in section B.3.c1, i.e. that the disclaimed prior art document "must then disappear from the prior art field to be taken into consideration", we emphatically disagree. It might be that in the fact situation underlying T 863/96 the Appeal Board 3.3.2 had reasons to take such a view on the allowability of disclaimers, but there is no history of case law by the Technical Boards (!) which would permit this restrictive interpretation in general. To the contrary, the Appeal Board 3.3.3 held in decision T 434/92- 3.3.3, "Molded articles/AMOCO CORPORATION", November 28, 1995, not published in the OJ EPO, in section 5.2 of the reasons:

"The function of a disclaimer is to excise a portion of the subject-matter of an already existing claim, for instance to exclude an area of prior disclosure. In this connection, the Board is not aware of anything in the cited decision T 0004/80 or elsewhere, which would justify a general requirement for there to be "no real relationship" of the disclaimer to the novelty destroying subject-matter (cf. decision under appeal, Reasons for the decision, para. 2.2.3.1).

Whilst the decision under appeal does not define precisely what is meant by "no real relationship", nevertheless if there were no relationship at all between the subject-matter of a claim and that of a disclaimer, the latter would clearly not succeed in its function of excising part of the former. Consequently, the fact alone that the subject-matter excised by a

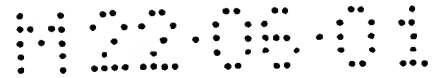
disclaimer is related in some way to that remaining after excision is not a justified objection to the introduction of a disclaimer." (emphasis added)

3.3.2 Riechmann (D36) does not come closest to the claimed invention

In this respect, we would like to note that as discussed above and acknowledged by the Opposition Division in its written decision, Riechmann (D36) does not disclose a general method for humanizing an antibody. Consequently, this document cannot necessarily be a document which comes closest to the claimed invention. It merely comes close to a result obtainable by the claimed method but not at all to the claimed method itself. This seems a subtle but is an important difference!

Accordingly, documents which generally address the problem of humanizing antibodies would have to be considered as the closest prior art. Notably, as summarized in section F.1. of the written decision, the Opponents when discussing inventive step of the anti-Tac antibody of the invention did not cite Riechmann (D36) as the document the person skilled in the art would have considered when trying to humanize a given antibody; see also the minutes at section 15. Thus, not even the Opponents were able to derive a teaching from Riechmann (D36) that could be applied to any other antibody.

For the above reasons, it is submitted that the claims of the new Main Request meet the requirements of the EPC.



4. AUXILIARY REQUEST I

As explained in section 2.2, *supra*, claim 1 of the First Auxiliary Request combines the subject matter of granted claims 7 and 8, thereby requiring that at least three substitutions are to be made in accordance with any one of rules (a) to (c). This feature positively distinguishes the subject matter of claim 1 from EP-A 0 328 404 (D48) and Riechmann (D36) which disclose one and two amino acid substitutions in the heavy chain of the humanized anti-Campath-1 antibody; see also section 3.2.3, *supra*.

As regards the meaning of the term "CDRs" in claim 1 of Auxiliary Request I we refer to section 3.1, *supra*.

In summary, claim 1 and dependent claims 2 to 14 of the first Auxiliary Request meet the requirements of the EPC.

5. AUXILIARY REQUEST II

As mentioned in section 2.3, *supra*, claim 1 of Auxiliary Request II corresponds to granted claim 7 but has been directed to the production of a humanized immunoglobulin light chain. Since EP-A 0 328 404 (D48) and Riechmann (D36) disclose amino acid substitutions only in the heavy chain of the humanized anti-Campath-1 antibody, the subject matter of claim 1 is novel over the teaching of those documents.

Furthermore, this restriction implies that the issue of whether or not reference to CDRs in this claims request could possibly represent an inadmissible broadening under Article 123(2) and/or 123(3) EPC is moot. That is because each of the three respective light chain Chothia "CDRs" or "hypervariable regions" (Chothia (D28)) is contained *within*

the respective Kabat CDR, so that "CDR's as defined by Kabat together with Chothia" means exactly the same as Kabat CDRs.

Therefore, the claims of Auxiliary Request II meet the requirements of the EPC.

6. ASPECTS OF PATENTABILITY

Aspects of patentability, i.e. enabling disclosure, novelty and inventive step are, of course, clearly in accordance with the submissions made by Patentee with its observations on April 7, 1998 and December 17, 1999. Further comments are unnecessary at this point.

7. SUMMARY

To summarize, in the opposition proceedings the Patentee has not been allowed to defend the patent on the basis of a claims request commensurate with the contribution of the invention as taught in the patent. With the amendments to the claims and the explanations given above, it is submitted that the patent meets the requirements of the EPC. Our request to set aside the decision of the Opposition Division and to maintain the patent on the basis of the new main request is therefore fully justified.



Dr. Hans-Rainer Jaenichen
European Patent Attorney

Encl.:

21 copies of the new Main Request
21 copies of Auxiliary Requests I and II
18 copies of the Appeal Brief

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Patentanwälte

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To the
European Patent Office
Munich

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(EP 95 10 5609.2)
Protein Design Labs, Inc.
HLBB File: GFOP 00 828 (Lit. No. 375)
Vossius & Partner Ref.: B 1060 EP/I

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July 13, 2001
Jae/PST/elh

Please be informed that our firm Vossius & Partner will act henceforth as co-representative of the Patentee in this case. Enclosed please find a corresponding authorization by the Patentee. However, please note that Hepworth, Lawrence, Bryer & Bizley and Mr. Richard Bizley specifically remain the address for service in this case. It is therefore respectfully requested that the European Patent Office should continue to direct all formal correspondence and copy correspondence to Mr. Richard Bizley.

Reference is made to Patentee's petition dated May 11, 2001 and to the Communication of Notices of Opposition dated September 4, 2000.

These are Patentee's observations pursuant to Article 101(2) and Rule 57(1) EPC to the oppositions against PDL's patent EP-B1 0 682 040 (hereinafter referred to as the "contested Patent") filed by

- I. Dr. Charles T. Harding
- II. Boehringer Ingelheim GmbH
- III. MedImmune Inc.
- IV. Schering Corporation
- V. Celltech Chiroscience Limited
- VI. Xoma (US) LLC
- VII. Novartis AG
- VIII. IDEC Pharmaceuticals Corporation

In the following, the parties are referred to as "Opponent I", "Opponent II", etc. . . .

In order to simplify reference to any documents, we enclose 9 copies of a consolidated list of all documents cited by the Opponents and by us in these observations (Annex I). Each of these documents is now given a consecutive number to aid reference by the Opposition Division.

1. INTRODUCTION

The invention claimed in the patent relates generally to the combination of recombinant DNA and monoclonal antibody technologies for the production of non-immunogenic antibodies and their uses. The method of the claimed invention enables a general approach toward the successful humanization of antibodies.

The prior art methods for producing humanized antibodies relied upon the presumed "self correcting" in assembling the CDRs on a new framework upon binding of the antigen; see, for example, Jones (D1), Verhoeyen (D2) and Riechmann (D3), all of which represent the work and strategy of the laboratory of Dr. Winter. As can be seen from those documents, the Winter laboratory preferred to use the framework from the variable region of the human NEWM antibody, the crystallographic structure of which was known,

see also Winter (D15) at page 17, 1st paragraph. It was their assumption that the framework of one and the same antibody can provide a passive scaffold onto which the CDRs from any other antibody may be grafted. Accordingly, when humanizing rodent monoclonal antibodies B1-8, D1.3 and YTH 34.5HL, respectively, Jones (D1), Verhoeyen (D2) and Riechmann (D3), all used the framework of the human NEWM antibody heavy chain as the acceptor, despite the fact that the donor antibodies had different antigen specificities.

The work of Riechmann (D3) did raise questions as to what extent the framework of a given antibody can provide a passive scaffold onto which the CDRs from another antibody may be grafted and retain their antigen binding affinity. However, in Riechmann (D3) antigen binding could be improved by particular considerations for the specific antibody investigated, so the general suitability of the acceptor heavy chain framework used, i.e., from the human NEWM antibody, was not questioned at all.

In contrast, the invention claimed in the contested Patent is based on the inventive concept that for producing humanized antibodies, the acceptor framework should be chosen to match the framework of the CDR donor antibody. Accordingly, when humanizing antibodies using CDR grafting, the claimed invention for the first time provided a criterion to select a framework which on its own is sufficient to retain antigen affinity, or for which at least the necessity of framework changes to retain antigen specificity is minimized. As shown in the post-published literature, it turned out that the chances of success with this method, i.e., that antigen specificity is retained with few or no framework substitutions, are considerably enhanced compared to the prior art approach. Hence, the method has been widely adopted.

Thus, the claimed invention contributes a method which on its own or optionally combined with further steps taught in the contested Patent leads toward the successful humanization of antibodies.

2. REQUESTS

2.1 General Requests

The Patentee requests that the oppositions be rejected and that the Patent be maintained on the basis of the new Main Request.

As an auxiliary measure, oral proceedings are requested in accordance with Art. 116(1) EPC. Furthermore, in order to expedite the proceedings, we request that an intermediate notice giving the preliminary view of the Opposition Division be issued in preparation for oral proceedings.

2.2 New Main Request

Claims 1 to 5 of the new Main Request correspond to claims 1 to 5 as granted.

Granted claim 6 has been deleted.

3. ALLEGED UNPATENTABLE SUBJECT-MATTER (ART. 100(A) EPC AND ART. 52 EPC)

Opponent II asserts that the claimed subject-matter comprises mental steps and therefore unpatentable subject-matter under Art. 52(2) EPC.

Opponent II's objection is unfounded. The claims relate to a method of producing a humanized immunoglobulin, i.e., to an industrial process, and therefore are of clear technical character. Opponent II seems to be of the opinion that steps 1 and 2 may be seen as mental steps which as such would not be patentable in view of Art. 52 EPC and that therefore the claims are unpatentable as well. However, there is no basis in the EPC for applying this so-called contribution approach for assessing patentability of a claimed subject-matter. The fact that one or more features of an otherwise claimed technical teaching relate to e.g. concepts, does not deprive the claim as such of technical character. In this context, we refer to recent decision T 931/95, "Controlling

pension benefits system/PBS PARTNERSHIP", dated September 8, 2000, not yet published in the OJ EPO, where the Board set out in catchword Nr. 4 that there is no basis in the EPC for distinguishing between "mental steps" of an invention and technical features when examining whether the invention concerned as a whole may be considered to be an invention within the meaning of Art. 52(1) EPC, thereby following the previous decisions T 1173/97, "Computer program product/IBM", OJ EPO 1999, 609.

In summary, there is no basis for the objection of Opponent II.

4. ALLEGED INADMISSIBLE BROADENING (ART. 100(C) EPC AND ART. 123(2) EPC, ART. 76(1) EPC)

4.1 Amended description

4.1.1 The term "CDR" in claim 1

Some of the Opponents allege addition of matter because of the paragraph [0012] of the printed specification of the contested Patent. They note that this passage was not in the patent application as filed and allege that it alters and adds to the meaning of claim 1.

The Patentee's position, as discussed in detail below, is that this passage was added only to provide the reader with additional scientific background on the structure of antibodies. It would have been clear to the skilled person that CDRs in claim 1 mean CDRs *as defined by Kabat*, a definition which is amply supported by the patent application. The passage does not put forward any definition of CDRs that combines the definitions of Kabat and Chothia; it merely notes that the Chothia definition is one way in which the term hypervariable regions or CDRs has been used.

That the cited passage does *not* in fact change the interpretation of CDRs in claim 1 from its intended meaning of CDRs according to the Kabat definition, may be seen from a large number of factors.

First, the added passage occurs only in the section of the Patent headed Background of the Invention, not in the Summary of the Invention nor the Detailed Description of the Invention nor the Experimental section. Importantly, nowhere does the contested Patent state that the Chothia definition is to be used in carrying out the invention or in understanding the claims.

Secondly, the Opponents to the EP-B1 0 451 216 patent asserted at great length, and the Opposition Division agreed in its Reasons for Decision (D50), that Chothia (D5) did *not* provide an alternative definition of CDRs but rather gave a definition of hypervariable loops. After reviewing all the evidence, the Opposition Division concluded, "... the overwhelming majority of publications referring to CDRs correctly use this term as defined by *Kabat*," ((D50) page 22, last paragraph), and, "There is no convincing evidence for an alternative or refined definition of *Kabat et al.* CDRs by *Chothia et al.*..." ((D50) page 23, first paragraph under d5). It follows from this reasoning that the skilled person reading claim 1 would necessarily give the word "CDRs" in claim 1 its scientifically standard meaning of *Kabat* CDRs, there being nothing in the claim indicating that it should have the *non-standard* meaning of Chothia CDRs.

Thirdly, the skilled person would be reinforced in this understanding by the way in which claim 1 refers to "a humanized immunoglobulin (Ig) having complementarity determining regions (CDR's) from a donor Ig combined with a framework region from human Ig acceptor light and heavy chains" (emphasis added). Of course, the skilled person would already know what a humanized immunoglobulin was from the work of Dr. Winter and colleagues, e.g., Winter (D15) and Riechmann (D3), which are referred to in the Patent. But the humanized antibodies described by Dr. Winter always combined the CDR's *according to Kabat* with a human framework region. Hence, taking into account that the contested Patent certainly does not purport to give a new definition of humanized antibodies, the skilled person would expect the

meaning of CDRs in claim 1 to match that already used by Winter when describing humanized antibodies, namely the Kabat definition.

Fourthly, and especially importantly, the use of the word "framework" in claim 1 necessarily implies that CDRs mean *Kabat* CDRs. That is because the term "framework region" is given a clear and precise definition in the contested Patent:

"As used herein, the term "framework region" refers to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved (i.e., other than the CDR's) among different immunoglobulins in a single species, as defined by Kabat et al., op.cit."; see Patent specification, page 6, lines 9 to 11 corresponding to page 12, lines 37 to page 13, line 3 of the divisional application as filed which is the identical paragraph of page 10, line 37 to page 11, line 3 of the application as originally filed.

Hence, the Patent unequivocally uses the *Kabat* definition for the framework region, i.e., the part of the variable region other than the Kabat CDRs. This same definition was given in the priority documents and the application as filed. But if the Kabat definition of framework is used, it only makes sense to also use the Kabat definition for the component with which the framework is to be combined, namely the CDRs. That alone would make it clear to the skilled person that CDRs in the claim must mean Kabat CDRs.

Finally, as extensively commented upon by the Opponents to the EP-B1 0 451 216 patent, other features of the specification make use of only the Kabat definition of CDRs. For example, in the construction of the exemplary humanized anti-Tac antibody, the donor CDRs that are transferred are defined according to Kabat (page 11, lines 28 to 29). Correspondingly, the CDRs underlined in the anti-Tac sequence are those of Kabat (page 4, lines 48 to 57, and Figs. 1 and 2 on page 16). Hence, the skilled person who turned to the experimental example in the contested Patent for guidance in carrying out the method of claim 1 would necessarily interpret CDRs according to the definition of Kabat.

In conclusion, for all these reasons, the term CDRs in claim 1 could only mean Kabat CDRs to the skilled person. Since the Kabat definition of CDRs was referenced in the patent application, there is no added matter in claim 1. Moreover, since the Kabat definition was also provided in the first and second priority documents, the contested Patent has priority to at least the second priority document, and in the view of the Patentee, to the first priority document as well.

4.1.2 The addition of references to prior art does not contravene Article 123(2) EPC

As explained in section 4.1.1, supra, the introduction of paragraph [0012] in the background section of the divisional application as filed does not provide a redefinition of "CDRs". It is a mere addition to the description of referenced prior art. This is already clear from the fact that the passage had been introduced into the background section of the specification. Furthermore, the paragraph in question at page 3, line 14 states that Chothia (D5) "have given an alternative definition of the hypervariable regions" based on the residues that constitute the loops in the three dimensional structures of antibodies. Indeed, the title of Chothia (D5) is "Canonical Structures for the Hypervariable Regions of Immunoglobulins". Thus, the discussion of Chothia (D5) in paragraph [0012] is an accurate representation of the teaching of Chothia (D5), although, like a few scientific publications, it also refers to the Chothia hypervariable regions as Chothia CDRs. The paragraph merely reflects that besides the original definition of the hypervariable regions that determine complementarity by amino acid sequence variability, an alternate definition of the hypervariable regions had been given based on structural considerations; see also the introduction of Chothia (D5).

From the case law of the Boards of Appeal, it is clear that such addition of discussion of background art to the description can not be interpreted as the addition of subject matter; see Headnote V of the leading decision

T11/82,"Control circuit/LANSING BAGNALL", OJ EPO 1983, 479. This principle was recently confirmed by decision T450/97, "Shampoo composition/PROCTER & GAMBLE", OJ EPO 1999, 67, where the Board explicitly stated in the Headnote that "the mere addition of a referenced prior art does not contravene Art. 123(2) EPC".

Thus, there is no basis for the objections of the Opponents.

4.2 Design versus production

Opponent II alleges that the parent application as originally filed relates to the "design" of humanized antibodies rather than to the production of such antibodies.

The Opponent's objection is unfounded. Already at the outset of the application as filed, it is stated that the invention relates generally to the production of a non-immunogenic antibody, see the application as originally filed at page 1, lines 4 to 8. Likewise, the application describes in great detail on page 16, line 12 to page 20, line 30 how recombinant DNA methods are used to produce the antibodies. The fact that the term "design" had been used in the original claims cannot of course change the fact that the originally disclosed methods of the invention comprise true process steps. Hence there can be no doubt that the application as filed relates to the production of humanized antibodies, which indeed is what the invention as described and claimed in the original application is all about.

For the above reasons, the objection of Opponent II is off the mark.

5. ENABLING DISCLOSURE (ART. 100(B) EPC AND ART. 83 EPC)

5.1 The claimed subject matter is sufficiently disclosed

5.1.1 The Patent provides sufficient guidance for obtaining humanized antibodies with high binding affinity

The Opponents have pointed out that the method of claim 1, comprising the selection of human acceptor frameworks that have at least 65% homology to the donor frameworks, is not generally *itself* sufficient to ensure high binding affinity of the humanized antibody. They have pointed to the Chung Declaration (D11), which was filed by the Patentee during opposition proceedings to EP-B1 0 451 216. This declaration reported that a version of the humanized anti-Tac antibody which had no donor substitutions in the framework did not have detectable binding affinity. In this regard, the Patentee agrees with the factual assertions of the Opponents, but not with their conclusions.

Claim 1 covers a *method* “comprising” certain steps, which implies that additional steps may also be used to yield an optimal product. The claim makes no assertion that the claimed steps alone are sufficient to produce a humanized antibody that has any particular affinity for antigen. The proper question in judging enablement is whether the method of claim 1 can in fact be carried out over the full scope of donor antibodies. Although there may be some minor quibbles on this point relating to the nature of the collection of human Ig chains and the comparison method used, which will be dealt with below, the Opponents have not seriously disputed this.

Claim 1 solves the technical problem over its full scope. That is, for *any* donor antibody, the claim provides a method of selecting the human acceptor light and heavy chain frameworks to be combined with the donor CDRs, so that after optional donor substitutions are made in the framework, the humanized antibody possesses therapeutically useful binding affinity while exhibiting acceptable immunogenicity in human patients. The

specification of the patent provides ample information, namely Criteria II – IV on page 7, by which those optional donor substitutions may be determined. In neither the opposition to the parent patent nor to the current patent, have the Opponents been able to provide *any specific example* of an antibody which could not be successfully humanized according to the teachings of the patent.

5.1.2 The claimed method is an advancement of CDR grafting

The principle of producing humanized antibodies by CDR grafting was known for example from Winter (D15) and its corresponding scientific publications Jones (D1) and Verhoeyen (D2), as well as Riechmann (D3). In this context, we would like to point out that the general CDR-only grafting method of Winter (D15) matured into a patent with substantially broad claims. Thus, the EPO was apparently of the opinion that Winter (D15) generally enabled the production of humanized antibodies by CDR grafting. Furthermore, although the Winter patent had been opposed, enabling disclosure was not a ground of the opposition. Thus, even the Opponent (Celltech, which is also Opponent V in the current proceedings) acknowledged enabling disclosure of the Winter patent (D15).

Moreover, the Jones (D1), Verhoeyen (D2) and Riechmann (D3) references are cited by the Opponents as closest prior art for the assessment of inventive step. In order to qualify as relevant prior art, a given document must of course provide an enabling disclosure. Documents (D15) and (D1), (D2) and (D3) according to the Opponents provide an enabling disclosure for the production of humanized antibodies at least to some extent. However, they have in common that they lack steps (1) and (2) of claim 1 of the contested Patent. These additional steps provide an improvement of the prior art methods for producing humanized antibodies, since as we will discuss below, the additional process steps considerably enhance the chances to obtain humanized antibodies with a useful binding affinity. In any case, it is untenable to argue that a method which comprises more process features than corresponding methods in the prior art is not enabled while the

prior art methods are. And as will be discussed in section 9, *infra*, the scientific community acknowledged the contribution of the method of the invention as providing a key step towards successful humanization of antibodies.

Hence, the Opponents' general objections to the claimed method for alleged lack of enabling disclosure cannot stand a closer scrutiny. The only issue that may be discussed in cases where an invention provides a further development of a method is whether the new process feature is enabled. Indeed, some of the Opponents made such objections to steps (1) and (2) of claim 1. However, as we will show below these objections are unfounded as well.

5.2 Alleged missing essential features

5.2.1 Alleged insufficiency with respect to homology

Opponent V (page 6) alleges that the skilled person could not perform the method of claim 1 because the patent provides no definition of homology.

In addition, several Opponents have alleged that the word "homology" in claim 1 is unclear. They assert that when the percent homology of two sequences is calculated, pairs of amino acids that have similar characteristics (i.e., are "homologous") are counted as matches and thus contribute to the percentage of homology. Since the contested Patent does not specify which pairs of amino acids are considered to be "homologous", they conclude that the term percent homology lacks clarity. Lack of clarity is not a ground of opposition, but this issue may be relevant to novelty, inventive step and sufficiency of disclosure.

It is the Patentee's position, which will be supported by multiple citations below, that when referring to sequences, "percent homology" normally *means* "percent identity," and the term is so used and understood by the vast majority of skilled persons. Hence, the question of which amino acids

should be counted as matches in claim 1 never arises; only *identical* pairs count toward the percent homology. Indeed, as also discussed below, in the context of the contested Patent, this would have been the only sensible interpretation of percent homology. The Opponents have noted that claim 1 and granted claim 6 of the Patent respectively refer to 65% homology and 65% identity, and they infer that the Patentee must have intended percent homology and percent identity to mean something different. *This inference is completely unwarranted.* Rather, the Patentee felt free to use percent homology in one place and percent identity in another place because he (and, as shown below, other skilled persons) consider them to be *synonymous*.

The Patentee's position is supported, in the first place, by the dictionary definition of homology as given for example in the standard reference work, Dictionary of Biochemistry and Molecular Biology (D51):

"sequence homology The identity in sequence of either the amino acids in segments of two or more proteins, or the nucleotides in segments of two or more nucleic acids"; see (D51), at page 437, first column.

Hence, homology between protein sequences is to be calculated by determining the identity of amino acids; nothing in this standard definition permits the inclusion of amino acids that are only similar.

This dictionary definition is in fact used by most scientists. As a first example, we cite Gorman (D52). As discussed in section 9, *infra*, this scientific publication is significant because it showed that the homology-based method of claim 1 was successful in humanizing a particular antibody, whereas the earlier method of Dr. Winter and colleagues was not. Gorman (D52) states:

"The V_H region of KOL was chosen because of all known human heavy chain V regions its overall amino acid sequence is very homologous to the Campath-9 V_H region (Fig. 1A) containing 72% identical residues ... By

contrast, the NEW V_H region sequence has only 47% identical residues. We reasoned ... we could maximize the chances of retaining correct CDR structure (and hence antigen affinity) by deriving framework sequences from a human V_H region that is most homologous to that of the rodent"; see (D52), page 4182, last paragraph, to page 4183, first paragraph (emphasis added).

This passage is not only an excellent restatement of the approach of the contested Patent, but clearly shows that Gorman (D52) uses percent identity to measure the extent of homology. Even more explicitly, Gorman (D52) later states:

"The KOL V_H region has a 72% homology to Campath-9 V_H region, whereas the NEW V_H framework has only 47% homology"; see (D52) at page 4184, first paragraph (emphasis added).

Hence, Gorman (D52) refers to the exact same sequences as having "72% identity" in one paragraph of their paper and having "72% homology" in another paragraph (and similarly for "47% identity" and "47% homology"). Beyond doubt then, the skilled authors of Gorman (D52) consider percent homology to be *synonymous* with percent identity, and feel free to use the terms interchangeably, in conformance with the Patentee's position.

As a second example, we refer to Chothia (D9), which was published in 1986 and has the same two authors as the Chothia (D5) paper where the Chothia definition of hypervariable regions was presented. In connection with the latter article, it has been observed that Dr. Chothia is an eminent scientist who is very careful about the meaning of the words he uses. So the following two passages respectively from the Results section and Conclusions section of Chothia (D9) are especially significant:

"Pairs whose sequence identity is > 50% have 90% or more of the residues of the individual structures within the common cores. Pairs whose residue identity drops to about 20% have common cores that contain between 42% and 98% of the residues of individual structures

(Table II, Figure 1)"; see (D9) at page 824, second paragraph (emphasis added).

"A protein structure will provide a close general model for other proteins with which its sequence homology is > 50%. If the homology drops to 20% there will be large structural differences that are at present impossible to predict"; see (D9) at page 826, first paragraph (emphasis added).

The text of Chothia (D9) (see, e.g. Table II) and the commonality of the key numbers 50% and 20% between these two passages makes it clear that Dr. Chothia uses "sequence identity" and "sequence homology" to mean *exactly the same thing*, that is, he calculates the percent homology by determining the percent of residues that are identical between two sequences, in accordance with the view of the Patentee.

To prove that the approach of Gorman (D52), Chothia (D9) and the Patentee was and still is the overwhelmingly preferred approach of those skilled in the art, we submit the Declaration of Mr. Wiesner, a specialist in bioinformatics (D61). Mr. Wiesner used a search of the National Library of Medicine Medline data base of biomedical journals to find scientific papers which use the term "percent homology" or related terms in their abstract. He then obtained these articles, which had publication dates from before the filing date of the contested Patent up to the current time, and studied them to determine what the authors meant by "percent homology".

As described in detail in his Declaration (D61), Mr. Wiesner analyzed 28 articles. In the vast majority of them – 25 – he found that the authors used percent homology to mean percent identity. In a few of these publications, the authors themselves explicitly or implicitly stated this. For the remaining papers, it was possible to prove that the authors used percent homology to mean percent identity by actually determining the percent identity between the relevant sequences, and showing it was equal to what the authors called percent homology. The fact that so many authors used the term percent homology to mean percent identity without even stating this in their papers



clearly demonstrates that they expected their readers to know that percent homology means percent identity.

Mr. Wiesner did find 3 articles where the authors defined percent homology to include pairs of amino acids that were similar but not identical. However, in each case, the authors specifically *stated* that they were using the term percent homology in this manner. This contrasts with the situation described above when percent homology means percent identity, which authors usually leave unstated. It is thus clear, not only that percent identity is the overwhelmingly preferred definition of percent homology, but it is the *default* definition. That is, percent homology means percent identity unless the author specifically provides a different definition. Notably, no such alternate definition of percent homology was given in the contested Patent. Hence, the skilled person would certainly have understood percent homology in claim 1 to mean percent identity.

To support a contrary position on the meaning of percent homology, Opponent I presents (page 12) the output of a sequence comparison made with the widely used BLAST 2 algorithm (D7). Opponent I refers to this as a "sequence homology plot" (page 12, paragraph 4.9.4) and notes that it includes matches of similar amino acids. However, what Opponent I fails to point out, but which is obvious from an inspection of (D7), is that nowhere does the BLAST 2 program present its output as "percent homology". The term "sequence homology plot" in reference to the BLAST 2 output is thus a creation of Opponent I – in fact, *the word homology does not even appear in (D7)*. Rather, BLAST 2 merely refers to the number of similar pairs of amino acids as "positives". Hence, all that Opponent I has really shown is that some scientists find this number to be of interest, and therefore a computer program has been written to determine it – which is in no way denied by the Patentee. What *is* vigorously denied by the Patentee is that skilled persons normally refer to the percent of similar amino acid pairs as percent homology. The Patentee's position on this is in fact *supported* by the absence of the word "homology" from the BLAST 2 output.

The same situation holds true with other widely used computer programs for comparing sequences. For example, the Wiesner Declaration (D61) includes output from the Wisconsin Package for sequence analysis (see (D61), Exhibit 3). As may be seen there, the percent of similar amino acids is (logically) called "Percent Similarity" – it is *not* called percent homology. It is also worth noting that when the percent of similar amino acids is of interest, the computer program generally inserts in the sequence alignment a special symbol between pairs of amino acids that are similar. For example, BLAST 2 inserts plus signs (see (D7)), whereas the Wisconsin Package inserts colons (see (D61), Exhibit 3). In striking contrast, the sequence alignments in the contested patent (page 16, Figs 1 and 2) have a symbol (a vertical line) *only between identical amino acids* (see the figure legends on page 4, lines 48 to 57). Indeed, the percent homology between the frameworks of the donor and acceptor sequences for humanized anti-Tac can be determined merely by counting the number of vertical lines outside the CDRs, and dividing by the total number of framework amino acids (giving the result $58/87 = 67\%$ for the heavy chain and $52/80 = 65\%$ for the light chain). Hence, if the skilled person had any remaining doubt about the meaning of percent homology in the contested Patent, the key Figure 1 would have made clear that it must mean percent identity.

For the above reasons, it is submitted that the meaning of "percent homology" in claim 1 is clear to the person skilled in the art.

5.2.2 Alleged insufficiency with respect to "collection"

Opponent VI (page 10) alleges that claim 1 is insufficient because it does not specify the collection of sequences from which the comparison is to be made. However, the person of skill in the art would certainly have been aware of such collections, for example, the comprehensive collection of Kabat (D4), a reference which is cited in the contested Patent. The Patent also specifically cites another collection that could be used, the National Biomedical Research Foundation Protein Identification Resource (page 6, lines 42 to 44), and provides yet further guidance by stating that typically the



collection will be "representative" and contain at least "10 to 20" distinct human heavy chains and similarly for light chains (page 6, lines 53 to 55). In fact, claim 1 could be carried out with any of these collections, and the skilled person would have no difficulty selecting one based on availability, convenience. etc. No more than that is required.

Opponent V (page 8) notes that different human antibody sequences might be chosen as acceptor depending on which collection was used for comparison. But so what? Any of the sequences selected according to the method of claim 1 would be satisfactory. Surely nothing requires that the method provide only a single solution to the problem of humanizing a particular donor antibody.

The decisive fact is that the contested Patent teaches which framework region to select as the homologous acceptor framework, and that such framework regions were available at the priority date of the contested Patent. The Opponents have not disputed this. If later a more homologous framework became available, then the person skilled in the art might prefer to select it, or to search other available data bases for comparable frameworks. In this context, we refer to the Decision in the opposition proceedings concerning European Patent EP-B1 0 549 581, where the Opposition Division had no doubt that the person skilled in the art had no problems with selecting homologous frameworks from any data base despite the fact that the number and content of such data bases change all the time; see section 7, last full paragraph on page 7 of the written decision concerning EP-B1 0 549 581 dated February 11, 2000.

5.2.3 Alleged insufficiency with respect to form of comparison

Opponent V (page 5 to 6) alleges that claim 1 is insufficient because it allows a choice of comparing frameworks or variable regions, and the skilled person would not have known which to choose. However, the mere fact that a claim envisages two alternative ways of performing a function certainly does not make it insufficient. In fact, either type of comparison would be

satisfactory, since comparing variable regions necessarily encompasses comparing the framework regions that are contained within them. And despite the Opponent's allegations, there is no ambiguity at all regarding what region must have at least 65% homology, since claim 1, step 2 specifies that the 65% homology be with "the respective donor framework sequences". The reason that the claim provides the option of comparing the entire variable regions is that many sequence data bases contain listings of variable regions rather than just their framework portions.

6. ENTITLEMENT TO DIVISIONAL STATUS (ART.76(1) EPC)

In section 4.8 of its opposition brief, Opponent I questions the divisional status of the contested patent because the paragraph [0012] was also present in the divisional application as published. In view of its submission under Art. 123(2) EPC, the Opponent concludes that the divisional application cannot be deemed to have been filed on the date of the parent application and that it is not entitled to any right of priority.

However, as discussed in detail in section 4.1.2, *supra*, the introduction of the paragraph in question does not amount to an inadmissible broadening but merely was an addition of reference to prior art in accordance with Rule 27(1) (b) EPC. As has been confirmed by the Technical Boards of Appeal, such addition of reference to prior art can be made at any time and even in a granted patent at the opposition stage without extending the disclosure beyond the content of the application as originally filed. Thus, Opponent I's submissions concerning entitlement to the divisional status are unsubstantiated for the same reasons as discussed in section 4.1.2, *supra*.

Accordingly, the divisional application and the granted patent are entitled to the filing date of the application as originally filed and enjoy the right of priority to the US applications filed on December 28, 1988 and February 13, 1989.

7. PRIORITY (ART. 87 AND 88 EPC)

As discussed in section 6, *supra*, the contested patent is entitled to divisional status and therefore is also entitled to claim priority of US applications US 290975 and US 310252 filed on December 28, 1988 and February 13, 1989, respectively.

Some of the Opponents object that the patent is not entitled to the first priority. Without conceding that this is correct, Patentee leaves this issue aside for the moment, since at present it is not relevant for the purpose of novelty and inventive step of the claimed invention.

On the other hand, it appears as if there is general agreement that the contested Patent enjoys the second priority date of February 13, 1989. For the sake of completeness, we refer to corresponding support for claim 1 in the second priority document; see, e.g., on page 3 the paragraph following the title "Summary of the Invention", and the discussion of criterion I starting at page 10 and continuing on page 11, the first full paragraph of which again explicitly recites the 65% homology criterion.

8. NOVELTY (ART. 100(A) EPC AND ART. 54 EPC)

As discussed in section 7, *supra*, the contested Patent and the claims are entitled to at least the second priority date, February 13, 1989. Consequently, the Patentee's parent patent application EP-A 0 451 216 and Queen (D6) do not belong to the prior art under Art. 54(1)(2) EPC. No further discussion of those documents is needed.

8.1 Novelty over Winter (D15) and Verhoeyen (D2)

Example 2 of Winter (D15) describes the humanization of the heavy chain of the D1.3 anti-lysozyme antibody; Verhoeyen (D2) is the scientific publication corresponding to this patent application and describes the same humanized heavy chain. Several Opponents have cited these references with regard to

novelty, asserting that the heavy chain frameworks of the mouse donor D1.3 and human acceptor NEWM antibodies are 65.5% or 66% identical, which is greater than the 65% criterion in claim 1.

First, even if this were true, these documents would not destroy the novelty of claim 1, which contains other essential features not found in Winter (D15) or Verhoeyen (D2). Specifically, claim 1 describes a method of producing a humanized immunoglobulin (Ig) in which the *light* chain as well as the heavy chain has a human framework, i.e., is humanized. However, neither Winter (D15) nor Verhoeyen (D2) discloses a humanized D1.3 light chain. Moreover, claim 1 specifies that the donor light and heavy chain sequences be compared with sequences in a collection of human Ig chains, but no such comparison is performed or implied in the cited documents, even for the heavy chain.

Furthermore, the assertion that the donor D1.3 and acceptor NEWM heavy chain frameworks are more than 65% identical is not correct. To make this clear, we have generated Exhibit 1 (D53) to these Observations, which compares the relevant sequences taken directly from Winter (D15). As can be seen from (D53) and its legend, the sequences are 64.4% identical, so do not have "at least 65% homology" as required by claim 1. The Opponents apparently derive a higher figure by considering the Q and X at the first positions of the respective sequences to be identical. However, this is absurd on its face: the symbols Q and X are not identical, and X is not a "wildcard" that can be matched to any other amino acid that the Opponents choose. (Rather, X means that the actual amino acid is unknown). Finally, putting the matter beyond doubt, the authors of Winter (D15) and Verhoeyen (D2) have *themselves* determined and published the number of matches between the D1.3 and NEWM frameworks, stating:

"Nevertheless, the grafting of hypervariable regions from mouse to human framework regions is sufficient to transfer the lysozyme-binding site, an extensive surface of interaction, despite the 31 of 87 residues that differ

between the heavy-chain framework regions"; see (D2) at page 1535, first paragraph (emphasis added).

Clearly, if 31 of 87 amino acids differ, then 87 minus 31, or 56, are the same, so the percent identity is $56/87 = 64.4\%$, which is completely consistent with the calculations of the Patentee.

8.2 Novelty over Riechmann (D3) and Clark (D14)

Riechmann (D3) describes the humanization of both the heavy and light chains of a rat antibody against the CAMPATH-1 antigen. Although the initial humanized antibody made in this way lost much of its binding affinity, this was largely restored by making substitutions in Chothia hypervariable loop H1. Clark (D14) is a patent application based on the same work.

These documents do not disclose the method of claim 1. Specifically, that method comprises in step 1 "comparing the framework or variable region amino acid sequences of the donor Ig light and heavy chains with corresponding sequences in a collection of human Ig chains." No such comparison is made in Riechmann (D3) or Clark (D14) for *either* the light or heavy chain. In fact, these documents only mention a *single* human heavy chain, that of the NEW antibody, not a collection of such chains. Moreover, the only heavy chain comparison made is between the donor and *reshaped* (humanized) chains, not the donor and *acceptor* chains (see legend to Fig. 1a on page 324 of Riechmann (D3), and the corresponding Fig. 2a of Clark (D14)). And while these documents mention two human light chains, NEW and REI, the NEW light chain was immediately dismissed "because there is a deletion at the beginning of the third framework region in NEW" (first paragraph of second column on page 325 of Riechmann (D3)). Hence, there is not even a hint that comparisons were made with the donor sequence before selecting the REI chain.

Finally, for completeness, we have ourselves compared in Exhibit 2 (D54) the heavy chain sequences of the rat donor antibody and human acceptor

antibody used by Riechmann (D3). As can be seen from (D54) and its legend, the framework sequences are only 53% homologous.

8.3 Novelty over Huston (D19)

Opponent V cites Huston (D19) against the novelty of claim 1. However, this citation is subject to the same objections as made above against Verhoeyen (D2) and Riechmann (D3). Huston (D19) does not describe any collection of human Ig chains but only presents again the human NEWM antibody. Still less does Huston (D19) disclose or even suggest that the donor mouse glp-4 sequences (page 40, third paragraph to page 41, second paragraph) be compared against the sequences in any such collection, as required by claim 1. Yet other arguments can be made against the relevance of Huston (D19) to novelty, for example, that Huston (D19) does not even disclose a humanized immunoglobulin, but only what he calls a biosynthetic antibody binding site (BABS), but these arguments are superfluous in light of the above.

9. INVENTIVE STEP (ART. 100(A) EPC AND ART. 56 EPC)

Any one of documents Jones (D1), Verhoeyen (D2), Riechmann (D3) and Winter (D15) may be seen as a candidate for the closest prior art. All these documents have in common that they teach to use the human NEWM antibody as source for the acceptor framework, at least for the heavy chain of their humanized antibody. However, no criteria have been taught how to select human acceptor frameworks other than those of the NEWM antibody or the one example with the REI antibody for the light chain in (D3). Furthermore, as is shown for example in Gorman (D52), the use of the NEWM based framework may result in very poor antigen binding.

The objective problem to be solved, in light of the methods disclosed in the prior art, can thus be considered to be the provision of further guidance in the process of humanizing antibodies, preferably improving the effectiveness of retaining antibody affinity.

A solution to this problem is provided by the method of claim 1, i.e., the use of a human acceptor framework the amino acid sequence of which has at least 65% homology with the framework sequences of the respective donor antibody. As already discussed in section 5, *supra*, the solution provided by the method of claim 1 has been generally acknowledged as means towards the successful humanization of antibodies. Furthermore, post-published documents such as Gorman (D52) demonstrate and confirm that the use of homologous human acceptor framework sequences maximizes the chances of retaining antigen affinity; see also section 9.6, *infra*. Accordingly, the problem has been solved by the invention claimed in the contested patent.

In the following we will show that none of the cited documents teaches or suggests to the person skilled in the art to perform steps (1) and (2) of claim 1 in order to solve the problem posed.

9.1 Inventive step over Jones(D1), Verhoeyen (D2) and Riechmann (D3)

Jones (D1), Verhoeyen (D2) and Riechmann (D3), which have been cited by the Opponents, were the three scientific papers presenting humanized antibody chains that were published before the priority date of the contested Patent. All three articles emanated from the laboratory of Dr. Winter. The Patentee submits that these papers practiced humanization in a consistent way that was quite different from the homology-based method claimed in the Patent, and which indeed taught directly away from any such method.

9.1.1 Jones (D1)

The first paper in this series was Jones (D1), in which the authors explained their choice of the human NEWM antibody heavy chain as acceptor this way:

“We grafted the CDRs from the V_H domain of the mouse monoclonal antibody B1-8 (ref. 7) into the V_H domain of

the human myeloma protein NEWM, whose crystallographic structure is known"; see (D1) at page 523, first full paragraph.

Hence, Jones (D1) apparently chose NEWM as acceptor because its 3-dimensional structure was known, and indeed that structure was extensively used in their paper (see, e.g., Fig. 1, which presents several stereo views of the structure). There is not the slightest mention that any other human antibody heavy chain was considered as acceptor, or that high homology was a factor in the choice of NEWM, or even of what the extent of homology between donor and acceptor frameworks actually was (it may readily be calculated to be 56%, well below the 65% criterion of claim 1).

9.1.2 Verhoeyen (D2)

Verhoeyen (D2) also utilizes the human NEW antibody for the acceptor and notes "the crystallographic structures of both parent antibodies are known ...," where the "parent" antibodies refer to the donor D1.3 and acceptor NEW antibodies ((D2), page 1535, second column). Again, there is not the slightest suggestion that the NEW heavy chain was selected from a larger collection based on its homology to the donor, or that the percent homology should be calculated or was in any way relevant. (Although Jones (D1) and Verhoeyen (D2) use the same human antibody as acceptor, unfortunately Jones (D1) refers to it as NEWM and Verhoyen (D2) as NEW. That these are in fact the same antibody is known because (i) the frameworks of the humanized chains in Fig. 2b of Jones (D1) and Fig. 2 of Verhoyen (D2) are identical, and (ii) Verhoeyen (D2) even references Jones (D1) as the source of the NEW heavy chain ((D2), page 239, last complete sentence of the second column)).

9.1.3 Riechmann (D3)

Finally, Riechmann again utilizes the "crystallographically solved" ((D3), page 325, second column) NEW antibody to provide the acceptor heavy chain, once again without consideration of any other human antibodies or of

homology. The authors' choice of antibody to provide the acceptor *light chain* is even more informative, since they state:

"The REI light chain was used because there is a deletion at the beginning of the third framework region in NEW"; see (D3) at page 325, second column (emphasis added).

The clear implication of this statement is that the authors would have *preferred* to use the NEW light chain, and did not do so only because of the deletion. However, the light chain frameworks of the donor antibody and the NEW antibody have a low homology of only 52%, whereas the light chain frameworks of the donor antibody and the REI-based antibody actually used as acceptor have much higher homology (as may readily be calculated after aligning the published sequences). Hence, Riechmann (D3) would actually have preferred to use the light chain with *lower* homology (below 65%), and was only prevented from doing so by the deletion. This shows beyond any doubt that Winter and his colleagues gave no consideration to the extent of homology, and that homology played no role in their method of humanization.

Where would this leave the skilled person attempting to humanize an antibody after the publication of the three prior art papers from Dr. Winter's laboratory, but before the teachings provided by the contested Patent? Such a person would necessarily turn to Dr. Winter's papers to learn to humanize an antibody – where else should he or she turn, no other antibodies having been at that time humanized? The skilled person would then learn that Dr. Winter's group had always used the *same* antibody to provide the human acceptor heavy chain – the NEW antibody. Moreover, each time, Dr. Winter and colleagues had been notably successful, albeit in Riechmann (D3) they needed to make a substitution in the Chothia hypervariable H1 loop to regain affinity. Surely, the person skilled in biotechnology, who is by definition conservative and cautious (T455/91 at section 5.1.3.3), would have followed the leaders in the field and used the same NEW antibody heavy chain rather than setting out to do something different. And even,

arguendo, had the skilled person wanted to use an acceptor antibody other than NEW, what he or she would have learned from the relevant prior art was that it is important to use an acceptor antibody whose crystallographic structure is known, *not* that the extent of homology has anything to do with the matter.

9.2 Inventive step over Winter (D15)

Winter (D15) is the patent application corresponding to Jones (D1) and Verhoeyen (D2); it presents no other examples of humanization. However, the Opponents have cited the following quotation from Winter (D15):

"It may be necessary only to transfer those residues which are accessible from the antigen binding site, and this may involve transferring framework region residues as well as CDR residues"; see (D15) at page 7, fourth paragraph.

To the extent that the skilled person might have learned anything from this rather brief and ambiguous remark, it would have been that it might be necessary to use *donor* amino acids in addition to the Kabat CDRs when humanizing an antibody. There is nothing whatever here that is relevant to how the *acceptor* antibody is chosen; in fact the entire text of Winter (D15) never suggests the necessity or even the desirability of considering acceptor antibodies other than NEW. The Opponents claim that Winter (D15) would have made it obvious to select, from available human antibodies, an acceptor with high homology to the donor. *But if it was obvious, why wasn't it obvious to Dr. Winter, who did no such thing?* It is absurd to suppose that it would have been obvious to one of ordinary skill in the art, when it wasn't obvious to the acknowledged leader in the field.

Indeed, after filing Winter (D15), when Dr. Winter and colleagues humanized another antibody in Riechmann (D3), they once again used the NEW antibody heavy chain as acceptor, although it only had 53% homology to the donor. Clearly, Dr. Winter himself had found no instruction in his own

patent application to select an acceptor framework with high homology to the donor. Even more conclusive is what the authors of Riechmann (D3) did when their initial attempt at humanizing the anti-CAMPATH-1 antibody lost most of its binding affinity. They did *not* even try making another humanized antibody using a more homologous framework. Rather, they made substitutions in Chothia hypervariable loop H1 that restored affinity. This procedure, rather than anything to do with homology, is all that the skilled person could have learned from the combination of Winter (D15) and Riechmann (D3).

9.3 Inventive step over Chothia (D5)

Chothia (D5) analyzed the structure of a number of antibodies, provided a new definition of hypervariable regions or loops, and proposed that these hypervariable regions could adopt only a limited number of canonical conformations. Certain Opponents infer that since these canonical conformations depend on particular amino acids in the antibody, it would have been obvious to the skilled person that these amino acids should be the same in the donor and acceptor antibodies, which could be achieved by choosing an acceptor with high homology to the donor.

However, what Chothia (D5) actually says is:

"The descriptions of the hypervariable regions given above suggest that their main-chain conformations are determined solely by particular residues within each region"; see (D5) at page 913, first paragraph of section 10.

Contrary to the assertions of the Opponents, this statement would clearly have told the skilled person not to worry about amino acids outside the hypervariable regions (i.e., in the framework), because the hypervariable regions already contain the information that determines their conformation. Moreover, to the extent that any amino acids in the framework do impact on the conformation of the hypervariable regions, these residues are highly

conserved between different antibodies, as pointed out in the Levitt Declaration ((D55), Section 15). For example, see the statements regarding conservation of residues in Chothia ((D5), page 907, first and second paragraphs). Hence, these amino acids would generally not be changed when humanizing an antibody, and would not be of concern to the humanizer.

These arguments are supported by the known relationships between the Winter and Chothia laboratories. Verhoeyen (D2) thanks Chothia and his co-author Lesk for discussions and comments (see (D2), page 239, footnote 23), and both Verhoeyen (D2) and Riechmann (D3) reference Chothia (D5). But as discussed above, neither Verhoeyen nor Riechmann suggest that it is preferable to use a more homologous acceptor. If Dr. Winter and his colleagues did not deduce this from Chothia (D5), is it believable that the person of *ordinary* skill would have done so? Similarly, but in the other direction, there is no statement in Chothia (D5) referring to humanization. And even the later paper Chothia (D10), published *after* Verhoeyen (D2) and Riechmann (D3), while providing further support for the original observations of Chothia (D5), again said nothing about humanization. If Dr. Chothia did not write that his work was relevant to humanization, *at the time*, why should the person of ordinary skill have deduced this?

9.4 Inventive step over Cheetham (D16)

Opponent V asserts (page 20-21) that the contested Patent lacks inventive step over Cheetham (D16) in view of Riechmann (D3) and Verhoeyen (D2). In response, it is to be noted first that Cheetham (D16) is a review article describing the earlier work of Jones (D1), Verhoeyen (D2) and Riechmann (D3); no new humanized antibodies or methods of humanization are presented by Cheetham (D16). While Cheetham (D16) comments that the work of Riechmann (D3) is in the realm of "tinkering" rather than "tailoring" ((D16), page 172, first line of second column), this remark does not go beyond what is already in Riechmann (D3), and certainly adds nothing to the skilled person's knowledge of how to humanize antibodies. At most

Cheetham (D16) alerted the skilled person that there may be difficulties in humanization, without making any contribution to solving them.

In fact, Dr. Cheetham wrote a 2500-word article reviewing all the then-published papers on humanization yet never suggested anything remotely related to the homology-based method of the contested Patent; the words "homology" or "identity" are not even used in Cheetham (D16). But Dr. Cheetham, who reviewed the field for a major journal, was certainly of at least ordinary skill in the art. Hence, the omission of any suggestion of the claimed method in Cheetham's important review article is powerful contemporaneous evidence that the method was *not* obvious to one of ordinary skill. This evidence is even further supported by another 1988 article, Verhoeyen (D56). That article, which was authored by two early workers in humanization, Verhoeyen and Riechmann, also reviewed humanization technique (page 76, third column) but again made no mention of homology. The only reasonable conclusion is that *Cheetham, Verhoeyen and Riechmann did not mention the relevance of homology in any of their papers because they did not think of it before the contested Patent, and neither would the ordinary skilled person have done so.*

9.5 Inventive step over Amit (D20), Sheriff (D21), Davies (D22) and Panka (D23)

Opponent V asserts that the contested Patent lacks inventive step over Amit (D20), Sheriff (D21), Davies (D22) and Panka (D23) in view of Riechmann (D3) because these papers suggest the importance of framework residues to antigen binding. However, in the opposition to the patent EP-B1 0 451 216, the Patentee submitted declarations from Dr. Panka, the first author of Panka (D23), and from Dr. Poljak, the senior author of Amit (D20), which are resubmitted here as Panka Declaration (D57) and Poljak Declaration (D58). These Declarants showed that their own papers were not relevant to humanization and were certainly insufficient to teach how to humanize antibodies, and Dr. Poljak made similar comments about Sheriff (D21).

Davies (D22) is a review article largely based on the work of Amit (D20) and Sheriff (D21) and goes no further.

Furthermore, even if these papers had suggested to the skilled person that some amino acid in the framework was important to humanization (which is denied), there is still a huge gap between any such notion and the homology-based method of the contested Patent. Indeed, choosing a more homologous acceptor antibody cannot even ensure that a desired amino acid will be found at any particular position in the framework sequence.

9.6 How those of skill in the art viewed the invention

Those skilled in the art clearly considered the method of the contested Patent to be new and important. For example, Dr. Adair, who at the time was employed by Celltech (a competitor of the Patentee and now one of the Opponents to the contested Patent) wrote the following in 1992:

"An alternative procedure for the identification of these important non-CDR residues has been described (Queen and Selick 1990). In this procedure, the murine variable region sequences are compared to available human sequences and the human sequence with the highest homology is taken as the acceptor framework....

This approach of using highly homologous human antibody sequences as the starting acceptor framework, and then also including, as necessary, other residues from outside of the CDR regions has been used successfully to humanize the anti-human CD3 antibodies YTH12.5 (Routledge et al. 1991) and UCHT1 (Shalaby et al. 1992), the anti-human CD4 antibody, CAMPATH-9 (Gorman et al. 1991), the anti-human CD18 antibody 1B4, (Daugherty et al. 1991, Law et al. 1992), the anti-T cell receptor antibody BMA031 (Kurrle et al. 1990, Shearman et al. 1991b), the anti-HTLV-IIIb antibody, 0.5 β (Maeda et al. 1991), the anti-human EGF receptor antibody, 425 (Kettleborough et al. 1991), and the anti-p185^{HER2} antibody, 4D5 (Carter et al. 1992b). ...

During the humanization of the 1B4 (Daugherty et al. 1991, Law et al. 1991), 0.5 β (Maeda et al. 1991), and

CAMPATH-9 (Gorman et al. 1991) antibodies, initial attempts to use the NEWM heavy chain, as described for the CAMPATH-1 humanization (Riechmann et al. 1988a), had proven unsuccessful in reconstituting binding activity"; see (D59) at page 16, last paragraph and page 17, first two paragraphs. The reference list of (D59) on page 37 lists the Queen and Selick 1990 citation as WO90/07861, which is the application from which the contested Patent descended.

This passage from an objective scientist is highly interesting for several reasons.

First, Dr. Adair here describes the homology-based method of the contested Patent as an alternative procedure to the method of Dr. Winter which was described earlier in his article. Moreover, Dr. Adair never suggests that this alternative procedure could have been derived from the earlier work of Dr. Winter or others.

Secondly, the passage makes clear that in 1992, only 2 years after publication of the patent application from which the contested Patent derives, the homology-based method was being widely used by many scientists. This rapid, widespread adoption of the method by the scientific community provides conclusive proof of its usefulness.

Finally, Dr. Adair points out several examples where the earlier method of Dr. Winter – which always utilized the NEWM heavy chain as acceptor – was unsuccessful in humanizing an antibody, while the homology-based method of the contested Patent was successful. For example, in Maeda (D60) cited by Dr. Adair, several different versions of a NEWM-based humanized heavy chain were made; see (D60), Fig. 2A on page 126 and the second paragraph under Results on page 129. However, none of these showed any binding to the antigen; see (D60) at page 130, first paragraph. In striking contrast, when a homology-based acceptor was used for the heavy chain, the humanized antibody had affinity within about 2-fold of the donor mouse antibody; see (D60) at page 130, second paragraph and page 131, first two paragraphs.

The Gorman (D52) paper, which was also cited by Dr. Adair, reports similar results. In fact, the principal observation of the Gorman (D52) article, which may have justified its publication in the prestigious journal *Proc. Natl. Acad. Sci. USA*, was that the homology-based method was highly successful for humanizing an anti-CD4 antibody, whereas the NEW-based method was not. This finding is highlighted in the abstract, introduction, results and discussion sections of Gorman (D52), for example:

"We have made two reshaped antibodies that differ only in their usage of human V_H region framework sequences, KOL and NEW, and found one form to be far superior to the other.... In this case then, it would seem that the selection of a human V region framework that was highly homologous to the rodent V region was the best strategy for framework selection. We have also successfully reshaped a CD3 antibody by the same approach (E.G.R., unpublished data), so this strategy may prove to be generally applicable to antibody reshaping"; see (D52) at page 4184, bottom of first column through first paragraph of second column (emphasis added).

This preference for the homology-based method of the contested Patent is especially noteworthy because Dr. Waldmann, the senior author of Gorman (D52), had earlier been a collaborator of Dr. Winter and a co-author of Riechmann (D3).

9.7 Summary

As has been demonstrated above, none of the cited documents teaches or suggests to consider the level of homology between the frameworks of the donor antibody and the acceptor antibody when humanizing antibodies. Thus, the claimed method of the present invention was not obvious to the person skilled in the art. Furthermore, as confirmed by post published art, the claimed method has been acknowledged as providing a general approach towards the successful humanization of antibodies. Accordingly, the claimed method solves the problem of providing a method for producing

humanized antibodies that enhances the chances of retaining antigen affinity.

Therefore, the method of claims 1 to 5 involve inventive step.

10. CONCLUSION

For the forgoing reasons, our request that the oppositions be rejected and the contested Patent be maintained on the basis of the enclosed new Main Request is fully justified.



Dr. Hans Rainer Jaenichen
European Patent Attorney

Encl.:

11 copies of a new main request
8 copies of the observations for the Opponents
9 copies of a list of cited documents (Annex I)
9 copies of documents D50 to D61
Authorization

DOCKET NO. CARP0001-100

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: Adair et al.

Confirmation No. 9631

Serial No.: 08/846,658

Group Art Unit: 1642

Filing Date: May 1, 1997

Examiner: Minh Tam B. Davis

For: HUMANISED ANTIBODIES

EXPRESS MAIL NO.: EY147 612 604US

Date of Deposit: March 17, 2003

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

SUPPLEMENTAL AMENDMENT

This paper is being filed following the helpful personal interview conducted on January 21, 2003. A Request for Continued Examination, with an accompanying Request for Reconsideration, was filed December 23, 2002 in response to the Final Rejection dated December 18, 2001, following a Notice of Appeal filed June 18, 2002, received by the Patent Office June 24, 2002. This amendment is supplementary to that request and presents arguments made during the interview.

Claims 24-31 were pending. All pending claims were rejected in the Final Rejection. A Request for Reconsideration was submitted May 20, 2002. An Advisory Action was mailed August 28, 2002. The Advisory Action indicated that the rejection of the claims under 35 U.S.C. §102(e) over U.S. Patent No. 5,585,089 (the Queen patent) was maintained. Applicants respectfully request withdrawal of this rejection in view of the documents submitted May 20, 2002, the Request for Reconsideration submitted December 23, 2002, and the following arguments.

In maintaining this rejection, it has been the Examiner's position that the Queen patent is entitled to the filing dates of its two earliest priority applications when used as a reference under 35 U.S.C. §102(e). Specifically, contrary to Applicants' position, the Examiner maintains that the priority applications in question provide written descriptive support for the phrase "outside

the Kabat and Chothia CDRs” in the claims as issued. The Examiner’s basis for this position is essentially a single sentence in the earliest filed application as follows:

The chains all exhibit the same general structure of relatively conserved framework regions joined by three hypervariable regions, also called CDR’s (see, “Sequences of Proteins of Immunological Interest,” Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Chothia [sic] and Lesk, J. Mol. Biol., 196:901-917 (1987), which are incorporated herein by reference.)

(Application Serial No. 07/290,975, “the ‘975 application,” paragraph bridging pages 9-10.) The Examiner argues that this sentence supports her interpretation that any reference to CDR in the claims of the Queen patent means the Kabat plus Chothia CDRs. Applicants maintain that this interpretation is inconsistent with Chothia and Lesk, the remainder of the ‘975 specification, the prosecution history, and, finally, the claims themselves.

First, although the quoted sentence cites the Chothia and Lesk reference, and incorporates it by reference, Chothia and Lesk does not support the Examiner’s interpretation that CDR means Kabat plus Chothia. Chothia and Lesk carefully distinguishes its “hypervariable loops” from Kabat’s CDRs.

The six loops, whose main-chain conformations vary and which are part of the antibody combining site, are formed by residues 26 to 32, 50 to 52 and 91 to 96 in VL domains, and 26 to 32, 53 to 55 and 96 to 101 in the VH domains L1, L2, L3, H1, H2, and H3, respectively. **Their limits are somewhat different from those of the complementarity-determining regions defined by Kabat et al. (1983) on the basis of sequence variability: residues 24 to 34, 50 to 56, and 89 to 97 in VL and 31 to 35, 50 to 65 and 95 to 102 in VH.**

(Chothia and Lesk, “Canonical Structures for the Hypervariable Regions of Immunoglobulins,” *J. Mol. Biol.*, vol. 196, p. 904, 1987, emphasis added.)

Second, the remainder of the specification of the '975 application indicates that references to CDRs are as defined as Kabat. For example, on page 10, line 2, the framework regions are defined in terms of Kabat (copy enclosed, Exhibit 1). If the framework regions are defined in terms of Kabat, the CDRs must be as well. On page 21 (copy enclosed, Exhibit 2), the protocol for selecting which residues in the heavy chain are to be donor is set out. At lines 19-22, residues which fall in positions within a CDR "**as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106**" are specified to be donor.¹ At lines 28-30, amino acid 30 is listed as a position **immediately adjacent to a CDR** to be changed to donor. Amino acid 30 is adjacent the first heavy chain Kabat CDR, but **within** the first heavy chain Chothia hypervariable loop. The description of Figure 1 of the '975 application indicates that it refers to the heavy chains and that the three CDRs are underlined (page 6, lines 1-6, copy enclosed, Exhibit 3). In Figure 1 (copy enclosed, Exhibit 4), only amino acids **31-35** are underlined for CDR1.

Third, Queen never so argued. In fact, rather than argue that CDR means Kabat plus Chothia, Queen added what ultimately became the recitation "outside the Kabat and Chothia CDRs" to overcome an obviousness rejection over, *inter alia*, the Riechmann reference. (See Paper No. 7, Application Serial No. 07/634,278, filed December 19, 1990, Exhibit 5, page 7.) The Riechmann reference discloses an antibody in which the CDRs (as defined by Kabat) and, additionally, residue 27 alone or residues 27 and 30 are changed to donor. The claims under rejection at the time recited that the CDRs "**and at least one residue immediately adjacent to at least one of said CDRs are from different immunoglobulin molecules than the framework regions.**" (Paper No. 6, Application Serial No. 07/634,278, filed December 19, 1990, Exhibit 6, page 1, emphasis added.) As noted above, the Kabat CDR1 for the heavy chain comprises residues 31-35. Residue 30 is, thus, immediately adjacent to at least one of the Kabat CDRs.

In response, the claims were amended to recite that the framework changes were "not in positions 26-30 of the heavy chain." (See Paper No. 10, Application Serial No. 07/634,278, filed

¹ The '975 application uses a linear numbering system. Accordingly, the CDR residues listed differ slightly from those listed in the foregoing passage cited from Chothia and Lesk, in which the Kabat numbering system was used.

December 19, 1990, Exhibit 7, page 3.)² Queen argued that the “[t]he position 27 and 30 modifications of Reichmann [sic] et al. were in fact within the Chothia-Lesk H1 CDR, rather than outside this CDR.” *Id.*, at page 17. Notably, Queen did not argue that the modifications were in the CDRs as those terms were used in the claims. The examiner, however, objected to the negative limitation language, because the example antibodies in the then specification showed changes at residues 27 and 30. The claims were then amended to recite framework changes “in addition to any such donor amino acids in positions 26-30 of the heavy chain.” (See Paper No. 17, Application Serial No. 07/634,278, filed December 19, 1990, Exhibit 8, page 2.) Again, Queen did not argue that residues 26-30 were already part of the CDRs as those terms were used in the claims. The rejection of the claims for obviousness over, *inter alia*, Riechmann was maintained. A continuation application was then filed. The claims were amended in a concurrently filed Preliminary Amendment to recite “outside the Kabat and Chothia CDRs.” (Paper No. 3, Application Serial No. 08/477,728, filed June 7, 1995, page 1.) The claims were allowed shortly thereafter.

Additionally, during prosecution of the parent of the application that issued as the Queen patent, a Glossary was submitted with an Appeal Brief which stated the following regarding the definition of Complementarity Determining Regions:

Two related but **distinct** definitions of the CDRs are in use – the **original** definition of **Kabat** (based on sequence variability), and the **newer** definition of **Chothia** (based on 3-D structure).

(Paper No. 24, Application Serial No. 07/634,278, filed December 19, 1990, page 1 of the Glossary, copy attached, Exhibit 9, emphasis added in part.)³ There is no mention of a “combined” definition.

² A Kabat plus Chothia CDR for CDR1 of the heavy chain would comprise residues 26-35 and, thus, would include residues 26-30.

³ To the best of the undersigned’s knowledge based upon a review of the prosecution history of the Queen patent, Queen did not argue that CDR meant Kabat plus Chothia in response to the rejection over Riechmann. Instead, Queen added the limitation that the changes were to be “outside the Kabat and Chothia CDRs.”

Finally, if CDR means Kabat plus Chothia as the Examiner maintains, the recitation in the claims “outside the Kabat and Chothia” preceding CDRs is superfluous. For example, see claim 1 of the Queen patent duplicated below.

1. A humanized immunoglobulin having **complementarity determining regions (CDRs)** from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^7 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside the **Kabat and Chothia CDRs**, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids:

- (I) is adjacent to a **CDR** in the donor immunoglobulin sequence or
- (II) contains an atom within a distance of 4\AA of a **CDR** in said humanized immunoglobulin.

(Claim 1, U.S. No. 5,585,089, emphasis added.) As is evident from the claim, three other passages within the claim refer merely to CDRs without further qualification. If CDR means Kabat plus Chothia as advanced by the Examiner, there would be no need to recite “outside the Kabat and Chothia CDRs” after the first reference to CDR because any framework changes to donor would be, by definition, outside the Kabat and Chothia CDRs.

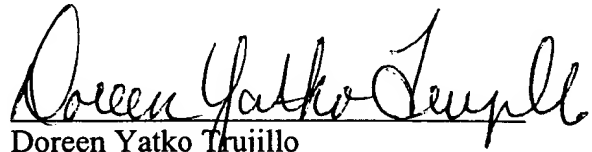
Applicants maintain that there is no written descriptive support in the two earliest filed applications for the requirement that the changes to donor be outside the Kabat and Chothia CDRs as recited in the claims as issued in the Queen patent. In further support thereof, the Examiner is directed to the patentees’ own admissions regarding the same as set forth in Applicants’ responses filed May 20, 2002 and December 23, 2002, discussion incorporated herein.

Applicants respectfully submit that the application is in condition for allowance and request declaration of an interference. If the Examiner disagrees, or feels a telephonic interview would be helpful, she is asked to contact the undersigned at 215-665-5593 to discuss.

Respectfully submitted,

Date:

3/17/2003


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RELATED PROCEEDINGS APPENDIX

NONE

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